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THE TRANSACTIONS OF THE ZOOLOGICAL SOCIETY OF LONDON

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QUANTITATIVE ANALYSIS OF NORMAL GROWTH OF THE PRONEPHRIC SYSTEM IN LARVAL AMPHIBIA

BY

H. FOX

Department of Zoology, University College, London

[Accepted 11th October, 1960]

(With 1 plate and 10 figures in the text)

The pronephric systems of young larvae of *Ambystoma mexicanum*, *Hynobius nebulosus*, *Cryptobranchus japonicus* and *Rana esculenta* were quantitatively analysed and compared with each other and with previous data of *Neoceratodus*. The morphological arrangement of the nephrostomial units was described. *Cryptobranchus* had the largest pronephric system among the urodeles. In terms of total larval length a comparison of the regression lines of a variety of pronephric components showed some of them to grow faster in *Cryptobranchus* than in axolotl, but the slopes of the regression lines of all components of *Hynobius* were not significantly different from those in axolotl. In all genera within the range of development studied the pronephric nuclear population, total pronephric tissue volume and overall pronephric volume increase with increase of larval length except in *Rana*, where the latter component measurement is stable from 17 mm. onwards. Glomus volumes and glomus external surface areas, except those for *Neoceratodus*, continue to enlarge. The pronephric cell volume size range is of a similar order in all genera except in *Cryptobranchus* larvae from 26 mm. to 32 mm. in length where it is considerably higher.

CONTENTS

	Page
Introduction	301
Material and methods	302
Morphological description	303
(a) Urodeles	305
(b) Anura (*Rana esculenta*)	309
Discussion	312
Summary	313
Acknowledgments	314
References	314
Explanation of plate	315

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CONTENTS

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in urodeles, *Rana* especially up to 17 mm. total length, and *Neoceratodus* with the exception of the glomus, are generally similar. Analysis further demonstrated that in urodeles in terms of total length there is a descending order of pronephros size, *Cryptobranchus japonicus* (the giant salamander) having the largest and *Hynobius nebulosus* the smallest pronephric systems, except for glomus measurements where the position is reversed for *Hynobius* and *Ambystoma*. Furthermore if it should prove that conditions of life and the metabolic properties of body tissues are generally similar in the urodele larvae, then as the *Cryptobranchus* pronephric system relative to body size is the largest, in addition to being morphologically the most primitive, it may well be per unit size functionally the least efficient.

In Amphibia as in *Neoceratodus* the pronephros is an evanescent structure and although it persists for the greater part of larval life (Cambar, 1949) it already shows signs of degeneracy in a 28 mm. *Eurycea bislineata*, and has completely disappeared in a 36 mm. specimen. It would appear to be in the process of degeneration in a 37 mm. *Cryptobranchus alleghaniensis*, and in a 34·5 mm. *Neoceratodus*, but well developed pronephroi are extant in a 37 mm. *Hynobius retardatus* and in a 46 mm. *Rana esculenta*.

The pronephric systems investigated in the present work are those which are still growing and would appear not to have started to regress, in a group of genera whose range of development in terms of total length is similar.

MATERIAL AND METHODS

Measurements were made upon various components of the pronephric systems of nine specimens of *Ambystoma mexicanum* (axolotl) ranging in total length from 10 mm. to 25 mm., five specimens of *Hynobius nebulosus* (14 mm., 17 mm., 20 mm., 22 mm., 32 mm. long), five specimens of *Cryptobranchus japonicus* (19·5 mm., 22 mm., 26 mm., 30 mm., 32 mm. long), and twenty-seven specimens of *Rana esculenta* in four groups of mean total length 8 mm., 12 mm., 17 mm. and 28 mm. (approximately) ; a total of thirty-eight urodele and fifty-four anuran pronephric systems. In the urodeles the data were compared by an analysis of covariance to allow for the variation in tadpole length, and regression, correlation and the absolute values of the various pronephric component measurements, obtained by a method of adjusted means, were calculated. The mean of each component measurement from the two pronephroi of each specimen was used. Comparisons were made between axolotl and *Cryptobranchus* and axolotl and *Hynobius*. The twenty-seven specimens of *Rana esculenta* were arbitrarily arranged in four successively older groups. Measurements of each group were compared with those of each succeeding group. The level of significance used throughout was $P<0{\cdot}05$.

All specimens were sectioned transversely at 10 microns and four specimens (representative of each group) of *Rana esculenta*, a 15 mm. specimen of *Hynobius*, a 16 mm. axolotl and a 27 mm. *Cryptobranchus* were sectioned horizontally at 10 microns in order to measure nuclear length at right angles to the transverse plane ; to correct the crude measurement of the pronephric nuclear population (Abercrombie, 1946). In all genera except *Cryptobranchus*

the mean pronephric nuclear length was about 12 μ: samples of 250 pronephric nuclei from each genus showed the mean nuclear length and standard error to be in axolotl 12·01 $\pm$0·095 ; *Hynobius* 11·70 $\pm$0·143 ; and *Cryptobranchus* 16·64 $\pm$0·169. The latter measurement is significantly different from those of the other two urodeles and the correction factor used in this case was 17 μ. The various pronephric nuclear length measurements obtained in *Rana esculenta* are shown in Table 3. Measuring techniques were identical to those used in previous investigations on the pronephros (Fox, 1956, 1957, 1959 b).

Axolotl and *Rana* larvae were killed in Smith's fixative and *Hynobius* and *Cryptobranchus* in Zenker's fluid. All specimens were paraffin wax blocked and stained in Ehrlich's haematoxylin and aqueous eosin, except for the 26 mm. and 30 mm. *Cryptobranchus* larvae which were stained with Heidenhain's Azan and the 32 mm. *Cryptobranchus* which was stained with Weigert's haematoxylin and counterstained with Pasini.

The axolotl and *Rana* embryos were grown in London tap water in the Department of Zoology, University College. The *Rana* eggs were obtained from a commercial dealer and Professor D. R. Newth generously supplied me with the axolotl eggs. The *Hynobius nebulosus* and *Cryptobranchus japonicus* specimens were from the collection of the late Professor J. P. Hill in the Department of Embryology, University College, London.

MORPHOLOGICAL DESCRIPTION

For an account of the development of the pronephric system in amphibia reference may be made to the works of Field (1891), Kerr (1919), and Goodrich (1930) ; for *Ambystoma punctatum* (Fales, 1935) and for *Cryptobranchus japonicus* (s. *Megalobatrachus japonicus*) (Mibayashi, 1928).

The pronephros in *Ambystoma punctatum* is first recognizable as a solid rudiment at a stage equivalent to Harrison stage 21–22 (approximately), and it is functional at about H 36 (Fales, 1935). Pronephric tubules are already formed in the 10 mm. axolotl, the youngest stage which was analysed, and are recognizable in a 9·3 mm. long *Cryptobranchus japonicus* (Mibayashi, 1928). A 15 mm. specimen of *Cryptobranchus* in my possession unfortunately damaged in the pronephros region had large yolk laden tubular pronephroi, open functional nephrostomes, ciliated nephrostomial tubules and a luminated pronephric duct which opened into the cloaca. The axolotl pronephros includes two nephrostomes (Pl. 1, fig. A), each one leading into a separate ciliated nephrostomial tubule which join to form the coiled collecting tubule continuous with the segmental or pronephric duct. Occasionally there are three nephrostomes and nephrostomial tubules (Field, 1891). All the pronephroi of the *Hynobius nebulosus* larvae including a slightly damaged unanalysed 11·5 mm. specimen possessed three open and functional nephrostomes and nephrostomial tubules (Pl. 1, fig. C), and both pronephroi of a 27 mm. *Hynobius retardatus* include four nephrostomes and tubules. A 37 mm. *Hynobius retardatus* possessed one pronephros with three and the other with four nephrostomial units for in the latter two nephrostomial units lead into a single second nephrostome. Examination of one 18 mm., two 19 mm. and one 21 mm. specimen of *Salamandrella* (*Hynobius*) *keyserlingii*

showed two specimens to have pronephroi like those of *Hynobius nebulosus*, one specimen had a similar pronephros but the third nephrostome of the other pronephros led into two nephrostomial tubules. The fourth specimen (19 mm. long) possessed one pronephros with three nephrostomial units and the other had four separate nephrostomes and nephrostomial tubules like both pronephroi of a 37 mm. *Cryptobranchus alleghaniensis*, the right pronephros of the 19·5 mm. *Cryptobranchus japonicus* and the left pronephros of the 22 mm. and the 32 mm. specimens. Five separate nephrostomes are recognizable in the left pronephros of the 19·5 mm. stage and in both pronephroi of the 26 mm. and 30 mm. specimens, but the fifth nephrostomial unit is always blind and vestigial and does not open into the rest of the tubular complex.

Table 1—Absolute size of group means of various pronephric components when adjusted for size of animal (total length). A. Overall mean total length for axolotl and *Cryptobranchus* combined, 20·3 mm. B. Overall mean total length for axolotl and *Hynobius* combined, 18·6 mm.

	A			B		
	Axolotl	*Crypto-branchus*	Percentage superiority of *Crypto-branchus* over axolotl	Axolotl	*Hyno-bius*	Percentage superiority of *Hynobius* over axolotl
Ant-post. length of pronephros (mm.)	0·528	1·176	+123	0·502	0·545	+ 9
Nuclear population of pronephros	2286	4092	+ 79	1999	2034	+ 2
Total volume of cells of pronephros (mm.^{3})	0·0245	0·0588	+140	0·0160	0·0109	−32
Total volume of lumina of pronephros (mm.^{3})	0·0119	0·0179	+ 50	0·0096	0·0052	−46
Overall volume of pronephros (mm.^{3})	0·0363	0·0767	+111	0·0255	0·0161	−37
Internal surface area of pronephric tubules (mm.^{2})	0·9424	1·3812	+ 47	0·7687	0·5521	−28
Calculated individual cell volume (μ^3)	9069	12810	+ 41	7942	5369	−32
Ant-post. length of glomus (mm.)	0·255	1·060	+316	0·251	0·303	+21
Total volume of glomus (mm.^{3})	0·0037	0·0308	+732	0·0022	0·0020	+ 6·5
Total external surface area of glomus (mm.^{2})	0·1260	0·7559	+500	0·1042	0·1186	+14

Furthermore the second and fourth nephrostomes in the right pronephros of the 30 mm. *Cryptobranchus* may have each divided into two separate ones to give rise to seven nephrostomes. The right pronephros of the 32 mm. specimen includes six separate nephrostomes which may be an expression of a more primitive ancestral numerical pattern or the result of division of the fifth nephrostome (Pl. 1, figs. D – J).

The pronephros of *Rana esculenta* six days after hatching (at a stage equivalent to Shumway stage 20 for *Rana pipiens*) consists of yolk-laden tubules which continue behind into a luminated duct that opens into the cloaca. Three open nephrostomes lead from the coelom into three ciliated nephrostomial tubules as in Group I (8 mm. specimens) and Group II (12 mm. larvae), but in the latter group the middle and posterior nephrostomes are situated close together. In Group III (17 mm. specimens) three pronephroi out of fourteen possessed three separate nephrostomes. The middle and posterior nephrostomes of the other eleven pronephroi communicated with the coelom by a common opening. In Group IV (28 mm. specimens) eight pronephroi are similar to the eleven pronephroi of the 17 mm. specimens and four pronephroi each include three nephrostomial tubules which open into a single common nephrostome (Table 4 and Pl. 1, fig. B).

Quantitative description

(*a*) *Urodeles*

In the covariance analysis between the various pronephric components of axolotl and *Cryptobranchus* the overall adjusted mean total larval length for the two groups combined was 20·3 mm. Means of various components of the pronephric system of *Cryptobranchus* showed significant superiorities over those of axolotl by the following stated percentages. Antero-posterior pronephric length (123 per cent) ; pronephric nuclear population (79 per cent) ; total pronephric tissue volume (140 per cent) ; overall pronephric volume (111 per cent) ; glomus length (316 per cent) ; glomus volume (732 per cent) ; glomus external surface area (500 per cent). Other component mean measurements though superior in *Cryptobranchus* were found not to be significantly different. Further analysis demonstrated that there is a significant difference in the slopes of the regression lines of the two groups in all measurements made, except for the pronephric nuclear population, total lumen volume, glomus length and glomus volume (Tables 1, 2). In terms of larval length the differences are shown in Figs. 1–10. Thus all the pronephric components except for the latter grow faster relative to body length in *Cryptobranchus* than they do in axolotl.

Similar comparisons between the various pronephric component measurements when the overall adjusted mean total larval length combined was 18·6 mm., showed significant percentage superiorities in axolotl over *Hynobius* for the means of the total pronephric tissue volume (32 per cent) ; total lumen volume (46 per cent) ; overall pronephric volume (37 per cent) ; calculated individual cell volume (32 per cent) ; but glomus length was significantly lower by 21 per cent. There are no significant differences in the slopes of the regression lines of the two groups for all measurements made, and it is evident

Table 2—Analysis of significance, regression and correlation of the various component

		Regression coefficient and standard error	t for difference between means adjusted by covariance analysis		t for difference in regression between		Correlation coefficients (r)
			A & C	A & H	A & C	A & H	
Ant-post. length of pronephros	A	+0·0014 ±0·00327					+0·1645
	H	+0·0009 ±0·00393		1·499		0·061	+0·1300
	C	+0·0234 ±0·00649	11·502		2·443		+0·9008
Nuclear population	A	+75·1 ±29·57					+0·6922
	H	+15·6 ±17·55		0·156		1·463	+0·4569
	C	+185·7 ±29·36	5·181		1·997		+0·9645
Total tissue volume	A	+0·0009 ±0·00020					+0·8637
	H	+0·0003 ±0·00025		2·550		1·747	+0·6096
	C	+0·0070 ±0·00098	2·755		3·107		+0·9720
Total lumen volume	A	+0·00050 ±0·000158					+0·7659
	H	−0·00005 ±0·000207		2·561		1·902	−0·1365
	C	+0·00149 ±0·000611	1·773		1·840		+0·8159
Overall pronephric volume	A	+0·0014 ±0·00034					+0·8412
	H	+0·0003 ±0·00044		2·556		1·858	+0·3325
	C	+0·0085 ±0·00131	2·739		3·027		+0·9666

A = axolotl ; C = *Cryptobranchus* ; H = *Hynobius*.

measurements between axolotl and *Cryptobranchus* and axolotl and *Hynobius* specimens.

		Regression coefficient and standard error	t for difference between means adjusted by covariance analysis		t for difference in regression between		Correlation coefficients (r)
			A & C	A & H	A & C	A & H	
Tubule internal surface area	A	+0·0371					
		±0·01049					+0·8012
	H	−0·0003					
		±0·01458		1·884		1·907	−0·0118
	C	+0·1164					
		±0·02963	2·075		2·350		+0·9149
Individual cell volume	A	+132					
		±84					+0·5122
	H	+116					
		±75		3·959		0·145	+0·6659
	C	+958					
		±165	2·033		2·814		+0·9583
Glomus length	A	+0·0024					
		±0·00152					+0·5096
	H	+0·0046					
		±0·00115		4·365		1·083	+0·9183
	C	+0·0038					
		±0·00947	17·942		0·198		+0·2253
Glomus volume	A	+0·00009					
		±0·000028					+0·7643
	H	+0·00014					
		±0·000012		0·543		1·513	+0·9897
	C	+0·00142					
		±0·000751	6·870		2·100		+0·7372
Glomus external surface area	A	+0·0034					
		±0·00115					+0·7694
	H	+0·0047					
		±0·00053		1·815		0·945	+0·9819
	C	+0·0190					
		±0·00294	19·006		2·956		+0·9659

A = axolotl ; C = *Cryptobranchus* ; H = *Hynobius*.

Table 3.—Various measurements of pronephric components of four groups of *Rana esculenta* larvae.

	Group I	Group II	Group III	Group IV
Number of specimens	7	7	7	6
Number of pronephroi	14	14	14	12
Mean total larval length (mm.)	8·0	12·0	17·0	27·7 ± 0·62
Mean nose-cloacal length (mm.)	2·47 ± 0·042	4·25 ± 0·109	5·67 ± 0·054	10·29 ± 0·245
Mean ant-post. length of pronephros (mm.)	0·367 ± 0·0083	0·449 ± 0·0096	0·483 ± 0·0099	0·547 ± 0·0133
Mean nuclear population of pronephros	895 ±27	2458 ±46	3418 ±109	4069 ±102
Mean total volume of cells of pronephros ($mm.^3 \times 10^{-3}$)	4·8 ± 0·13	16·6 ± 0·61	24·3 ± 1·23	28·8 ± 1·01
Mean total volume of lumen of pronephric tubules ($mm.^3 \times 10^{-3}$)	1·9 ± 0·25	14·0 ± 0·99	22·5 ± 1·39	22·2 ± 0·89
Mean overall volume of pronephros ($mm.^3 \times 10^{-3}$)	6·8 ± 0·32	30·6 ± 1·47	46·9 ± 2·51	51·0 ± 1·64
Mean internal surface area of tubules ($mm.^2$)	0·2400 ± 0·00719	0·9614 ± 0·04198	1·3415 ± 0·05658	1·4502 ± 0·05017
Mean calculated individual cell volume of pronephros (μ^3)	5445 ±213	6718 ±149	7064 ±171	7083 ±184
Mean length of nuclei at rt. angles to T.S. Section (μ)	11·59 ± 0·148	11·59 ± 0·121	11·50 ±0·123	11·51 ± 0·132
Number of glomi	14	14	14	12
Mean ant-post. glomus length (mm.)	0·192 ± 0·0104	0·174 ± 0·0025	0·174 ± 0·0040	0·206 ± 0·0055
Mean total volume of glomus ($mm.^3 \times 10^{-3}$)	0·96 ± 0·099	1·19 ± 0·061	1·42 ± 0·074	1·78 ± 0·101
Mean total external surface area of glomus ($mm.^2$)	0·0538 ± 0·00393	0·0624 ± 0·00202	0·0694 ± 0·00273	0·0871 ± 0·00372

Table 4—Measurements of the nephrostomes in the four groups of *Rana esculenta* larvae.

	Group I	Group II	Group III	Group IV
Number of specimens	7	7	7	6
Number of pronephroi	14	14	14	12
Mean total larval length (mm.)	8·0	12·0	17·0	27·7 ±0·062
Mean nose-cloacal length (mm.)	2·47 ± 0·042	4·25 ± 0·109	5·67 ± 0·054	10·29 ± 0·245
Number of nephrostomes	42	42	42	36
Diameter of anterior nephrostome base (mm.)	0·0493 ± 0·00165	0·0507 ± 0·00165	0·0557 ± 0·00173	
Diameter of middle nephrostome base (mm.)	0·0543 ± 0·00137	0·0600 ± 0·00182	0·0671 ± 0·00384	
Diameter of posterior nephrostome base (mm.)	0·0350 ± 0·00174	0·0336 ± 0·00133		
Total diameter of nephrostome bases (mm.)	0·1386 ± 0·00231	0·1443 ± 0·00310	0·1228 ± 0·00438	0·1217 ± 0·00725

that pronephric growth in term of larval body length is similar in the two groups of genera (Figs. 1–10, Tables 1 & 2).

In *Cryptobranchus* there is a high degree of correlation for all the pronephric component measurements, except for glomus length, with increase in total larval length. Similar but less marked correlations were found in axolotl, except for antero-posterior pronephric length. In *Hynobius* there is little correlation for pronephric length, total lumen volume, overall pronephric volume and tubule internal surface area, but correlation coefficients for other components were generally similar, or in the case of glomus length, glomus volume and glomus external surface area higher than in axolotl (Table 2).

(*b*) *Anura* (*Rana esculenta*)

Comparisons between groups I and II (8 mm. and 12 mm. specimens), groups II and III (12 mm. and 17 mm. specimens) and between groups III and IV (17 mm. and 28 mm. [approximately] specimens) of *Rana* showed that there were successive and significant superiorities in the older over the younger larvae in the means of the following measurements by the stated percentages, which are given in the same order as the above comparisons. Nose-to-cloacal length (72 per cent, 33 per cent, 81 per cent) ; antero-posterior pronephric length (22 per cent, 7·6 per cent, 13 per cent) ; pronephric nuclear population (175 per cent, 39 per cent, 19 per cent) ; total pronephric tissue volume (246 per cent, 46 per cent, 18·5 per cent). Comparisons between

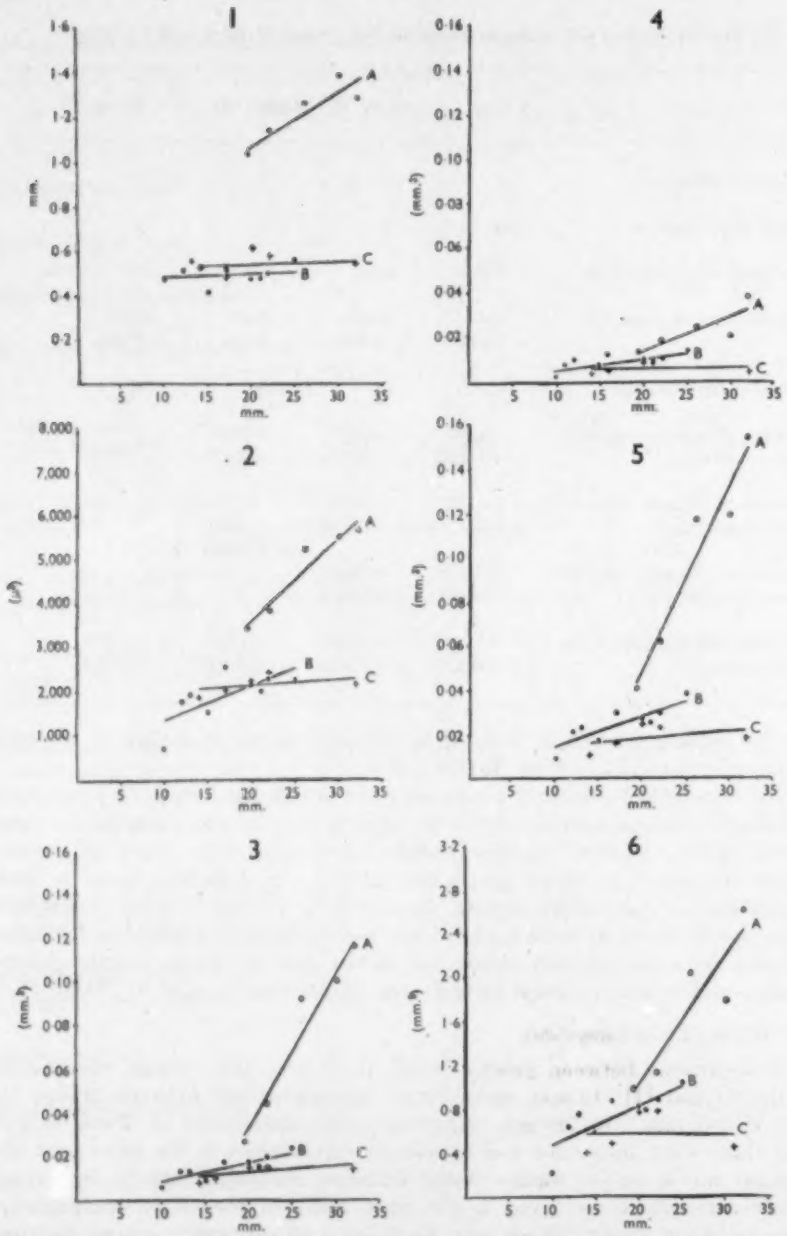

Fig. 1—Regression lines of the antero-posterior length of the pronephros related to the total larval length in mm.: A (open circles) represents ten *Cryptobranchus* pronephric components (5 means), B (closed circles) represents eighteen axolotl pronephric components (9 means) and C (crosses) represents ten *Hynobius* pronephric components (5 means).

Fig. 2—Regression lines of nuclear population of the pronephros related to the total larval length in mm. Scheme is identical to Fig. 1. [*continued next page*]

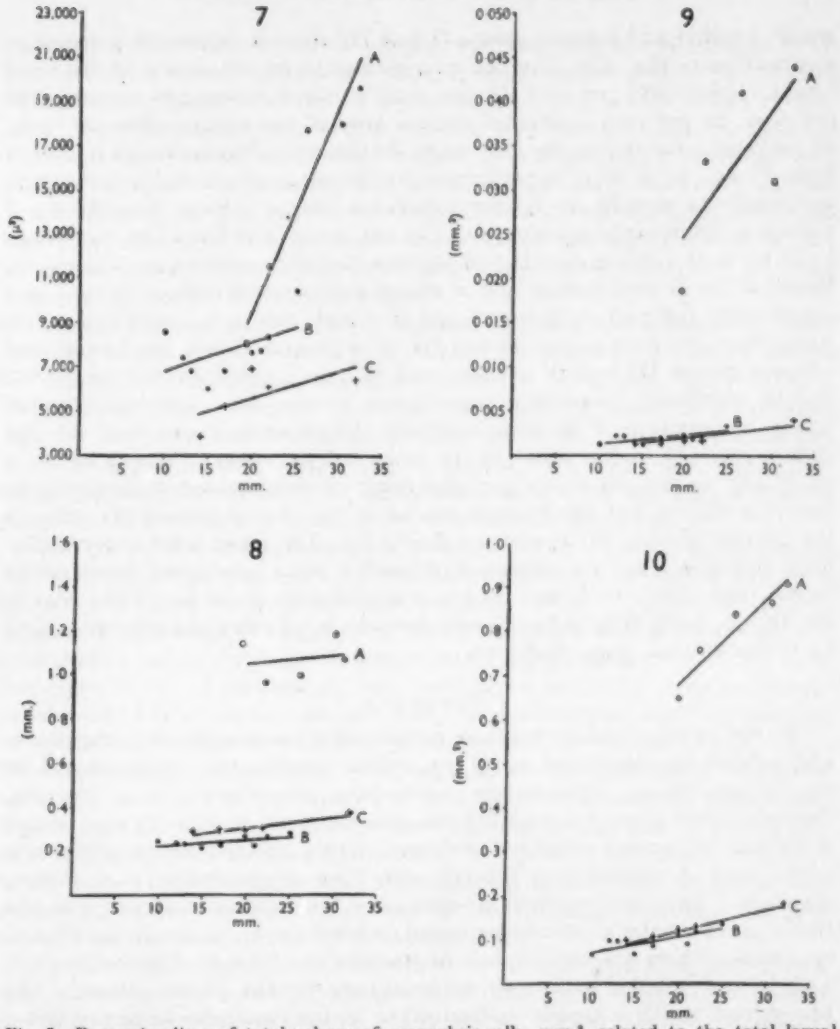

Fig. 3—Regression lines of total volume of pronephric cells, mm.³, related to the total larval length in mm. Scheme is identical to Fig. 1.

Fig. 4—Regression lines of total volume of the tubule lumina, mm.³, related to the total larval length in mm. Scheme is identical to Fig. 1.

Fig. 5—Regression lines of overall volume of the pronephros (volume of cells and lumina together), mm.³, related to the total larval length in mm. Scheme is identical to Fig. 1.

Fig. 6—Regression lines of internal surface area of the pronephric tubules, mm.², related to the total larval length in mm. Scheme is identical to Fig. 1.

Fig. 7—Regression lines of pronephros individual cell volume, μ³, related to the total larval length in mm. Scheme is identical to Fig. 1.

Fig. 8—Regression lines of length of glomus, mm., related to the total larval length in mm. Scheme is identical to Fig. 1.

Fig. 9—Regression lines of glomus volume, mm.³, related to the total larval length in mm. Scheme is identical to Fig. 1.

Fig. 10—Regression lines of external surface area of the glomus, mm.², related to the total larval length in mm. Scheme is identical to Fig. 1.

groups I and II and between groups II and III showed significant percentage superiorities in the older over the younger larvae for the means of the total lumen volume (640 per cent, 61 per cent) ; overall pronephric volume (350 per cent, 53 per cent) ; internal surface area of the tubules (300 per cent, 40 per cent) ; but during the time when the mean total larval length increased from 17 mm. to 28 mm. (approximately), the means of the latter pronephric measurements showed no further significant change. Mean individual cell volume is significantly superior in the 12 mm. group over the 8 mm. specimens by 23 per cent ; it then does not change significantly as development proceeds. Means of the glomus volume and of the glomus external surface area are not significantly different in groups I and II (8 mm. and 12 mm. specimens), but comparisons between groups II and III (12 mm. and 17 mm. specimens) and between groups III and IV (17 mm. and 28 mm. [approximately] specimens) showed significant percentage superiorities in the older over the younger larvae in the means of the glomus volume (19 per cent, 25 per cent) and the glomus external surface area (11 per cent, 25·5 per cent). Glomus length is practically unchanged throughout the range of development studied, except that it is slightly but significantly smaller in the 17 mm. (Group III) than in the 28 mm. (Group IV) specimens (Table 3). The mean total nephrostome base diameter does not change significantly when specimens increased in length from 8 mm. to 12 mm. but it is significantly lower by 15 per cent in the 17 mm. group than in the 12 mm. specimens. It then remains unchanged up to the 28 mm. stage (Table 4).

DISCUSSION

In the 10 mm. axolotl incipient mesonephric blastemata are recognizable and tubules are developed at 12 mm. which possess open nephrostomes in the 13 mm. stage. Mesonephric blastemata are present in a 19·5 mm. *Cryptobranchus japonicus* and tubules are incipient in the 22 mm. stage. A 14 mm. *Hynobius nebulosus* possesses darkly stained mesonephric cells which have developed into tubules with open nephrostomes in a 17 mm. specimen. Early mesonephroi are seen in a 9 mm. *Rana esculenta*, with the first signs of tubules at 10 mm. and open coelomic nephrostomes in the 12 mm. specimen. There are mesonephric blastemata in a 14 mm. *Neoceratodus* and well formed tubules with open nephrostomes by the 16 mm. stage. The pronephros, which is usually understood to be the functional kidney of larval vertebrates, is functional in all these genera before mesonephric cells are recognizable, yet pronephroi and mesonephroi presumably function simultaneously for a considerable period of larval life until the pronephros suffers degeneration, and it may well be more correct to classify the amphibian pronephros as the main functional kidney of early larval stages.

The somewhat similar patterns of early pronephric growth in urodeles may be compared with the results obtained in *Rana* and with the more limited information available (range 11·5 mm.–16 mm. total length) for *Neoceratodus* (Fox, 1960). In the range of development studied, with increase in larval length the pronephric length tends to increase in *Cryptobranchus* and *Rana* but not in axolotl, *Hynobius* and *Neoceratodus*, and the pronephric nuclear

population and total volume of pronephric tissue continue to enlarge in all genera. Total lumen volume and total internal surface area of the tubules enlarge in the urodeles except for *Hynobius*, and in *Rana* up to the 17 mm. stage, but the mean total lumen volume does not significantly alter in *Neoceratodus*. Overall pronephric volume increases steadily in every case except for *Rana* where it is stable from the 17 mm. stage onwards. Glomus length tends to increase slightly in urodeles but does not change in *Rana* and shortens in *Neoceratodus*. Glomus volume and glomus external surface area do not change in *Neoceratodus* but enlarge in all the urodeles and in *Rana* from the 12 mm. to the 28 mm. stages.

Mean individual pronephric cell volume is practically constant in *Rana* from the 12 mm. stage onwards (cell volume size range 6700–7080 μ^3) as it is in specimens of *Hynobius* ranging from 20 mm. to 32 mm. (6100–6900 μ^3), *Neoceratodus* from 11·5 mm. to 16 mm. (6450–6700 μ^3) and *Cryptobranchus* from 26 mm. to 32 mm. total length (17,800–20,600 μ^3). The cell volume size range in axolotl larvae from 10 mm. to 25 mm. total length was found to be more variable (6000–10,500 μ^3). Nevertheless except for the high values in *Cryptobranchus* there is a similar order of cell size in all groups.

The pronephros of *Hypogeophis* originates from about a dozen segmentally arranged units, though the last three do not become fully developed, and there are relics of others in front and behind (Brauer, 1902). The two pronephric tubules in axolotl (and *Triturus*) or *Neoceratodus* are merely survivors of a once greater series of tubule rudiments extending along the side of the body (see Kerr, 1919). Anura usually have pronephroi each with three pronephric tubules and nephrostomes—at least early in development—and occasionally an extra third nephrostomial unit is recognized in axolotls (Field, 1891). Three pronephric tubules and nephrostomes are recognizable in *Hynobius nebulosus* and four in pronephroi of a 19 mm. *Salamandrella* (*Hynobius*) *Keyserlingii* and 27 mm. *Hynobius retardatus* and in the pair of pronephroi in a 37 mm. *Cryptobranchus alleghaniensis*. Often four, but usually five, and in one case six nephrostomes and nephrostomial tubules were present in the pronephroi of *Cryptobranchus japonicus* larvae, two more than the four first reported in the same species by Mibayashi (1928).

Hynobius and *Cryptobranchus* are primitive urodeles (Fox 1954, 1959 a) and demonstrate their primitive organization in the retention of many chondrocranial and branchial ancestral structures. It is thus of interest, though not wholly unexpected, that relics of ancestral pronephric structures should be recognized in these genera to shed further light upon amphibian organic evolution.

SUMMARY

1. The pronephric systems of collections of young larvae of axolotl (*Ambystoma mexicanum*) (10 mm.–25 mm. long), *Hynobius nebulosus* (14 mm.–32 mm. long), *Cryptobranchus japonicus* (19·5 mm.–32 mm. long) and *Rana esculenta* (8 mm.–28 mm. long) were quantitatively analysed. Comparisons were made between the measurements obtained in (*a*) axolotl and both *Hynobius* and *Cryptobranchus*, (*b*) successively older arbitrarily chosen groups of *Rana*, (*c*) the urodeles, *Rana esculenta* and previous data of *Neoceratodus*.

2. Consideration was given to the segmental arrangement of the nephrostomes and nephrostomial tubules in the amphibia investigated.

3. The pronephric system of *Cryptobranchus* was found to be the largest among the urodeles. The regression lines of a variety of pronephric components were compared, in terms of total larval length, and some components grow faster relative to body length in *Cryptobranchus* than they do in axolotl. There are no significant differences in the slopes of the regression lines of axolotl and *Hynobius* for all components measured.

4. In all genera the pronephric nuclear population, total pronephric tissue volume and overall pronephric volume increase with increase in larval length within the range studied, except in *Rana* where the overall pronephric volume is stable from 17 mm. onwards. Glomus volume and glomus external surface area do not change in *Neoceratodus* but continue to enlarge in amphibia. Cell volume size range is of a similar order in all genera except for *Cryptobranchus* where in larvae from 26 mm. to 32 mm. long it is considerably higher.

ACKNOWLEDGMENTS

Thanks are due to Professor M. Abercrombie and Professor P. B. Medawar who generously gave their comments and advice. Mr C. Atherton prepared the photomicrographs and Mr H. C. Barker assisted in the preparation of several specimens of *Cryptobranchus japonicus*.

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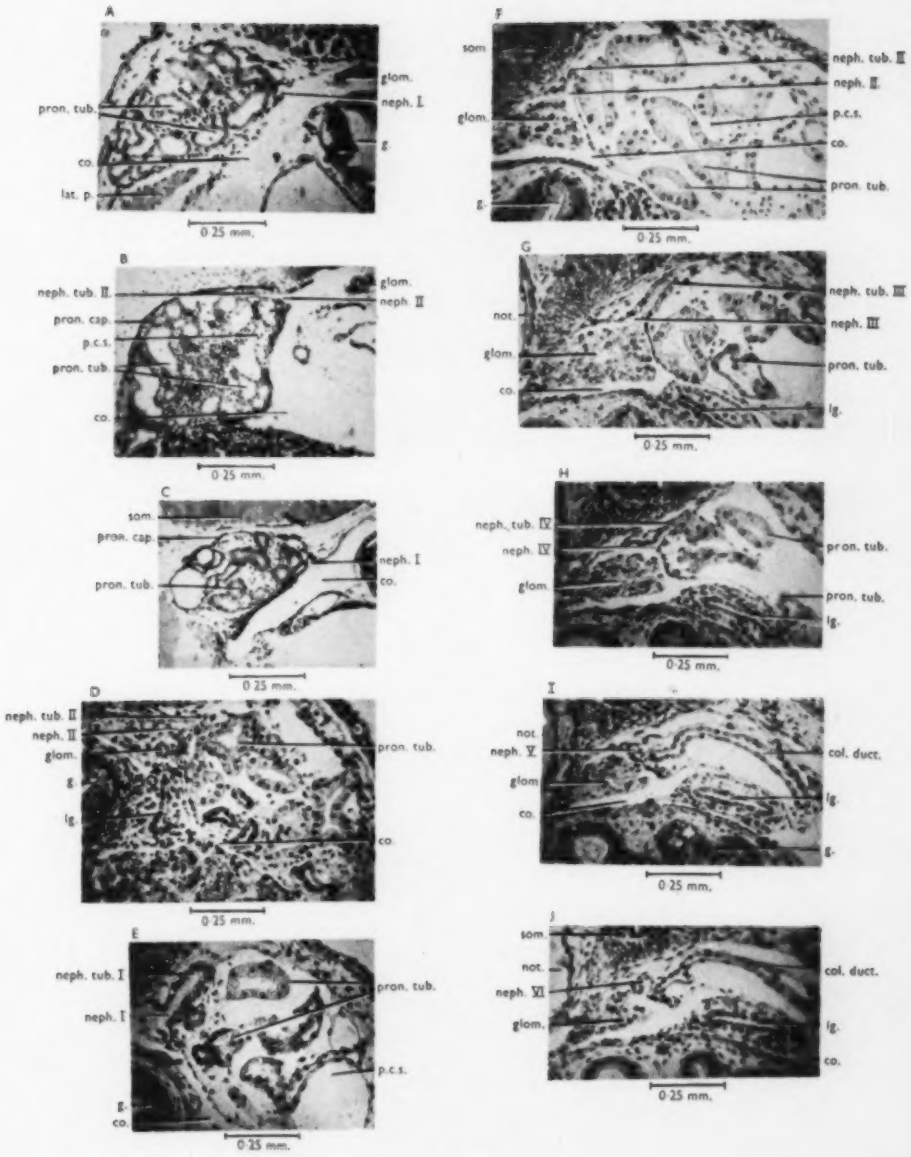

Quantitative analysis of normal growth of the pronephric system in larval Amphibia.

EXPLANATION OF PLATE

All sections are transverse and 10μ thick.

Fig. A.—Axolotl 25 mm. long, nose-to-cloacal length 13·35 mm. Section through nephrostome I, 0·22 mm. from anterior end of left-hand side pronephros. Length of pronephros 0·57 mm.

„ B.—*Rana esculenta* 28 mm. long, nose-to-cloacal length 10·39 mm. Section through middle nephrostome and nephrostomial tubule (nephrostome II) 0·29 mm. from anterior end of left pronephros. The three nephrostomes are confluent. Length of pronephros 0·57 mm.

„ C.—*Hynobius nebulosus* 32 mm. long, nose-to-cloacal length 14·40 mm. Section through nephrostome I, 0·16 mm. from anterior end of the left pronephros. Length of pronephros 0·54 mm.

„ D.—*Cryptobranchus japonicus* 26 mm. long. Section through nephrostome II, 0·49 mm. from anterior end of right pronephros. Length of pronephros 1·27 mm.

„ E.—*Cryptobranchus japonicus* 32 mm. long. Section through nephrostome I, 0·18 mm. from anterior end of right pronephros. Length of pronephros 1·32 mm.

„ F.—Specimen and pronephros as in Fig. E. Section through nephrostome II, 0·53 mm. from anterior end of pronephros.

„ G.—Specimen and pronephros as in Fig. E. Section through nephrostome III, 0·94 mm. from anterior end of pronephros.

„ H.—Specimen and pronephros as in Fig. E. Section through nephrostome IV, 1·15 mm. from anterior end of pronephros.

„ I.—Specimen and pronephros as in Fig. E. Section through nephrostome V, 1·24 mm. from anterior end of pronephros.

„ J.—Specimen and pronephros as in Fig. E. Section through nephrostome VI, 1·28 mm. from anterior end of pronephros.

co., coelom ; col. duct, collecting duct ; g., gut ; glom., glomus ; lat. p., lateral plate mesoderm ; lg., lung ; neph. I–VI, nephrostomes I–VI ; neph. tub., nephrostomial tubule ; not., notochord ; p.c.s., post-cardinal sinus ; pron. cap., pronephric capsule ; pron. tub., pronephric tubule ; som., somite.

THE RED GORAL OF THE NORTH-EAST FRONTIER REGION

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[Accepted 11th October 1960]

(With 1 plate)

Reports dating back to 1912 of a remarkable foxy-red coloured goral or goat-antelope (*Nemorhaedus*) from S.E. Tibet and N.E. Assam have been investigated. In 1931 Lord Cranbrook collected a specimen of this goral in the Adung Valley, Upper Burma, and recently Mr H. L. Cooper has presented to the British Museum (Natural History) a rug made of skins of three of these gorals taken in 1922 in the Mishmi Hills of Upper Assam. Study of these specimens has led to the proposal of a new species of *Nemorhaedus* based not only on colour but also on comparative size and proportions. A colour plate of skins of the new animal is given.

CONTENTS

	Page
Introduction	317
Nemorhaedus cranbrooki sp. n.	319
General remarks	322
References	323
Addendum	324

INTRODUCTION

The genus *Nemorhaedus*, containing the goat-antelopes or gorals of the Himalaya and the mountains of Tibet, Assam, Burma, China and Korea, is considered by Ellerman and Morrison-Scott (1951), following G. M. Allen (1940) to contain only one species, *Nemorhaedus goral* Hardwicke, with several recognizable local races. All these, with one exception, are of a rather uniform type of colouration which only varies moderately in the geographic races. The coat, rather shaggy and thick, is generally grey, greyish fawn or greyish brown in colour, often with a notable grizzling of black and frequently with a blackish dorsal line and some black on the fore limbs. There is generally a white or creamy throat patch. The local variations do not obscure the obvious unity of nearly all the forms in one species. The exception, *Nemorhaedus goral baileyi* Pocock from south-east Tibet, is a short-haired uniformly warm brown animal, which will be discussed below.

In 1931 the Earl of Cranbrook, while making zoological collections in the Adung Valley, Upper Burma (in the extreme upper basin of the Irrawaddy) in company with Capt. F. Kingdon Ward, obtained a single specimen of a remarkably differently coloured goral which was examined and commented on by the late Capt. Guy Dollman of this Department. In a report on the joint collection, Dollman (1932, 1932 a) referred to this specimen as a new goral which he intended to name after Lord Cranbrook, but no description of any kind was given at the time nor was any formal name actually proposed.

In an appendix to this report Lord Cranbrook and Capt. Kingdon Ward added some comments on the distribution of the new goral.

The description of the new goral, and of other smaller mammals in the same collection believed to be new, was never proceeded with, and the specimen, B.M. no. 32.11.1.170, remained undescribed in the National Collection. Possibly the lack of further material to confirm the discovery led to hesitation in committing a description to print, for in the 1935 edition of Rowland Ward's " Records of Big Game, African and Asiatic Sections ", 10th edition, edited in part by Dollman, there is no mention of this striking goral.

Very recently, however, striking confirmation of the existence of a hitherto undescribed form of goral from the general region of Upper Assam and Upper Burma has come from an unexpected source.

Mr H. L. Cooper of Guernsey, formerly of the Indian Forest Service, submitted to the British Museum for identification a rug made of three skins of an unidentified animal. He informed me (*in litt.*) that these skins were from goat-like animals killed in March or April 1922 in the Mishmi Hills of Upper Assam, on the north side of the Lohit River above Sadiya (at that time headquarters of the North-east Frontier District). At the time Mr Cooper was hunting Takin, and shot one at a salt lick on the watershed between the Digaru (Dibang of most maps) and Tiddim Rivers, at a height of about 6–7,000 feet, about two days' march up a ridge on the left bank of the Digaru.* This would appear to be about 28° 30′ North, 96° East. Grazing on a hill slope above the salt lick were a number of moderately sized goat-like animals, of which four or five were killed by Mr Cooper's Mishmis for meat. Mr Cooper describes the horns as being shortish, perhaps four to six inches long, black, curving obliquely back without any convolutions or excrescences. Skins of three of the animals were later cut into rectangles without heads or limbs, and sewn together to make a rug. It is this rug which has now been sent to London for examination.

The general colour of each skin is a bright foxy-red, with a more or less distinct black dorsal line. The fur is long, soft and shaggy. When these skins were compared with Lord Cranbrook's goral from the Adung Valley it was obvious that the animals were identical, and that the colour difference from any other goral was most marked.

Further, during the course of investigations into the fauna of this relatively little-known part of the world, I have traced previous references to the occurrence of a foxy-red goral in this area. Lt-Col F. M. Bailey, C.I.E., who travelled extensively in this frontier region before the first World War, has mentioned animals of this type. In Bailey (1912) he says, p. 344, " I had noticed that the people of Sanga Chu Dzong and at several other places wore coats made of the skins of goral of a foxy-red colour, which were said to come from the neighbourhood of Rima. I made enquiries about this, and was told that these animals were plentiful in the hills downstream from Rima."

Sanga Chu Dzong is shown on Bailey's map at about 29° 12′ North, 97° 10′ East, on the Tsai-lung Chu branch of the Zayal Chu, in south-east Tibet at about 11,700 feet. Rima is lower down the Zayal Chu (which later joins the

* See Cooper (1923) for description of the locality.

Lohit River) at about 28° 12′ North, 97° 12′ East, in extreme south-east Tibet, altitude 4,839 feet.

Col Bailey informs me (*in litt.*) that " several foxy-red goral were shot in the Mishmi Hills in the expedition there in 1913. These were obtained in the vicinity of Mipi (on the Dibang River) and the valleys to the east. Say approximately 95° Long., 29° North Lat. I did not get one myself but I saw some. I can't remember much about the Foxy Goral seen worn as coats at Sanga Chu Dzong." Further, " I noted that the Goral near Rima were foxy-red—I did not get or even see a specimen, but the people wore coats made of these red skins ". The latter observation was published in Bailey (1913), where, in a report on mammals and birds in south-east Tibet, he says, " In addition to the above small mammals, Takin and Goral were shot. One of the latter which was obtained at Ho-kou (on the Yalung River at approximately 101° East, 29° 30′ North) was of a greyish colour, while near Rima the Goral is a foxy-red and skins of these animals are used for making coats ".

From the above references, taken in conjunction with the evidence of the Cranbrook skin from the Adung Valley and with the Cooper skins from the Mishmi Hills, it seems clear that the goral of this frontier region is of a distinct type, hitherto unnamed. I propose that it be named

NEMORHAEDUS CRANBROOKI, sp. n.

Type. Male adult skin, with left ramus of mandible. B.M. no. 32.11.1.170. Collected by Lord Cranbrook, 27th February, 1931, at 8,000 feet in the Adung Valley, Upper Burma, 97° 40′ East, 28° 10′ North. Original number 185. The skull was kept, but cannot at present be traced, and only the left ramus of the mandible is available.

Diagnosis. A goral of comparatively small size, distinguished immediately from all other forms by the bright foxy-red colour of the greater part of the head, body and limbs above and below. A blackish dorsal stripe from the nape nearly to the root of the mainly black tail. No pale throat or chest patch. Ears shorter than in other forms.

Description of type. General colour above bright foxy-red, the dorsal body hairs about two inches long, basally light brown, becoming paler, tipped broadly with rich red. The overall hue calls to mind the colour of the maned wolf, *Chrysocyon jubatus*, of South America, in its reddest coat, and also to some extent the Indian wild dog, *Cuon javanicus*. Muzzle and cheeks less rufous, more tawny, small blackish patch above rhinarium, anterior margin of upper and lower lips whitish, posterior margins of lips and a small interramal patch dark brown. The longer red hairs of the crown are black-based, and between the bases of the horns the hairs are mostly all black, with a small but distinct cluster of pure white hairs. Outer surface of ears mainly fawn, slightly grizzled with blackish, inner surface white. Ear margins dark brown, with basal inch of outer lobe white. Black of the crown and nape continuing as a narrow spinal line of blackish brown hair becoming dark reddish on the rump. Tail hairs long, blackish at the base of the tail with some red tips, the terminal hairs all black forming a tuft five inches long. Colour of the

whole of the throat and chest is chestnut red, paler than the dorsal surface. There is no paler throat or chest patch as in other gorals. A narrow blackish area on the centre of the chest, the remainder of the undersurface a rather bright tawny buff, becoming white on the scrotum. Limbs coloured as upper surface of the body, becoming rather golden towards the hoofs. No black anywhere on the limbs, but the underside of the tarsus rather brownish.

The skins forming the rug submitted by Mr Cooper are incomplete, each being a rectangle of the body skin completely lacking any of the limbs as well as the head and neck and tail. But sewn into the rug are three sections of fore limb skin. Allowing for the fact that the skins may have undergone some fading, the rug having been used for some time in India, they agree closely with the type. There is some variation in the distinctness of the dorsal line, the largest skin having a very distinct black line from the shoulders to the rump, while in the smallest of the three the dorsal line is barely perceptible. The portions of leg skin appear to be identical in colour with the legs of the type.

It is a pleasure to record that Mr Cooper, on being informed of the special scientific interest of the skins, at once offered to donate them to the National Collection, and I take this opportunity of expressing the Museum's appreciation of his generous action.

Measurements. Flesh measurements of the type, taken in the field : head and body 975 mm. (39″), tail 100 mm. (4″), hind foot 200 mm. (8″), ear 100 mm. (4″). Height at shoulder 575 mm. (23″), height at rump 600 mm. (24″), girth round chest 625 mm. (25″). Mandible : total length from front of incisors to condyle 153·4 mm., toothrow (crowns) 66·4 mm.

Cranial characters. The absence of the skull hampers comparison, but the complete left mandible, with all teeth intact, shows that the animal was fully adult. Features of the dentition are the smallness and narrowness of the outer incisiform tooth, and the narrowness of the cheek teeth, with a poorly developed terminal lobe of m_3. The mandible shows a marked reduction in length as compared with mandibles of *Nemorhaedus goral* having a toothrow of about the same length, so that the whole skull could be presumed to be short in proportion.

Distribution, habitat, habits. The field label of the type bears the note "Uncommon. A pair seen on precipice in jungle." Cranbrook in Dollman (1932 a) reported that this goral did not appear to come down much below 8,000 feet, and in the summer goes up into the open above the tree line (12,000 feet) ; the ordinary Himalayan species range downwards to 3,000 or 4,000 feet. According to the natives the goral does not cross the main range, but is probably common on all the hills above 8,000 feet in this area, as far south as the hills in 'the Triangle' east of Fort Hertz. Capt. Kingdon Ward added that it was fairly widely distributed on the Burma and Assam frontiers. Bailey's mention (1913) of a grey goral being obtained at Ho-kou, on the Yalung River (101° East, 29° 30′ North) no doubt refers to *Nemorhaedus goral griseus* Milne-Edwards, and is an indication that the red goral is confined to a comparatively limited area. So far as is at present known, its distribution lies,

	Head and body	Tail	Hindfoot	Ear	Skull, greatest length	Upper toothrow	Mandible	Lower toothrow
N. cranbrooki, Type ♂	975	100	200	100	—	—	153·4	66·4
N.g. baileyi, Type ♂	1068	81 ?	c. 250	106	208	60	—	—
N.g. goral W. Himalaya ♂	—	—	—	—	204	63	—	—
N.g. goral ♂	—	—	—	—	202	66	163	67
N.g. goral	—	—	—	—	—	—	169	66
N.g. goral	—	—	—	—	—	—	175	66
N.g. goral ♂	—	—	—	—	210	67	169	66
N.g. hodgsoni Nepal ♀	1125	150	262	125	—	—	—	—
N.g. griseus Szechuen ♂	1110	200	258	149	223	69	179	71
N.g. griseus Szechuen ♂	1084	158	235	136	207	68·8	175	72·5
N.g. griseus Ichang ♂	—	—	—	—	200	66	168	69
N.g. griseus W. China ♂	—	—	—	—	213	67	176	72
N.g. griseus Shensi ♂	—	—	—	—	217	65·5	186	68·5
N.g. griseus Lower Burma ♂	—	—	—	—	200	62	164	62
N.g. griseus Yunnan. Minima and maxima of 4 ♂, 5 ♀ (Allen, 1940) ♂	1000–1060	130–150	260–280	117–140	184–210	60–66	—	64–70

Measurements in millimetres

very approximately, within the co-ordinates 96° to 98° East longitude, 27° to 29° 30′ North latitude.

The evidence of Mr Cooper regarding the red gorals encountered by him in 1922 in the Mishmi Hills refers to a remarkable tameness, or perhaps absence of fear of man as such, to such an extent that his Mishmis were able to kill four or five without any difficulty. He informs me (*in litt.*) "I certainly saw the head Mishmi walk up to one and put the barrels of my shot gun against its side, and shoot it with a so-called lethal bullet. The others were probably killed in the same way or with daos or stones. . . . I suspected they were a rare animal as the Mishmis did not seem to know them and they were so tame, whereas the takin were very wild and fled at our approach. . . . The part of the hills where I found them is, or was, a no-man's-land between the Mishmis of the Delai Valley and the Abors to the west of the Dibang. It is a very rough country."

This is a most interesting report, and in the absence of further knowledge it is hardly justifiable to offer any comment, other than to suggest that possibly long isolation and freedom from native hunting of that particular goral population had led to lack of awareness of man as a predator. There is no evidence of similar behaviour in the red goral noted by Bailey, nor in any other goral, which in general have the reputation of being very wary and difficult to approach.

GENERAL REMARKS

The colour distinction of this new goral is obvious, and in addition its comparatively small size, more particularly as shown in the mandible and ear length, indicates that the animal is likely to represent something more than a colour variation, which would in any case be of a type completely unknown in other gorals. The mandible is clearly shorter than in the Himalayan *N. goral goral* or the Burmese and Yunnan *N. g. griseus*, although the toothrow is not reduced in length. The only other form with which more detailed comparison is required is that described by Pocock (1913) as *N. baileyi*, but listed by Ellerman and Morrison-Scott (1951) as *N. goral baileyi*. This is known only from the type skin and skull collected by Col. Bailey on 3rd July 1913, at Dre, Yigrong Tso, Po Me, south-eastern Tibet, north-west of Shoma on the Yigrong, a tributary of the Tsangpo or Upper Brahmaputra. Bailey's map (1914) shows Dre at about 95° East, 30° 25′ North. This skin is readily distinguished from all other races of *N. goral* by its warm brown ungrizzled coat of rather short hair, and by its well-developed white patches on throat and chest. Since the locality is not a great distance in air miles from the Mishmi Hills the question naturally arose as to the possibility of *baileyi* representing the summer coat phase of the red goral. The Cranbrook skin was taken in February, the Cooper skins in March or April, so that we have no summer specimen.

There are, in my view, several reasons why the two may be considered quite different animals. First, in other gorals there does not appear to be such a great contrast in coat colour or length and texture as would be indicated by the supposition that *baileyi* and *cranbrooki* represented the summer and winter

coat respectively of the same species. Secondly, the terrain in which *baileyi* was collected is a considerably drier region than that in which *cranbrooki* is known. Thirdly, *baileyi* is distinguished externally by the large white chest patches and a white throat patch, whereas *cranbrooki* has no suggestion of light patches anywhere in this area. Fourthly, there is a clear size difference between the two, *baileyi* being notably larger both in skin and skull measurements; taking the mandible of *cranbrooki* against the skull, without mandible, of *baileyi*, it is obvious that the skull of *cranbrooki* would have been much shorter.

It is noteworthy that there appear to be no reports or specimens of any of the grey or greyish brown gorals, *N. goral*, from the area occupied by the red goral. To the west, in the Himalayan range, are *N. goral goral* and *N. g. hodgsoni*, to the east and also apparently to the south as far as Thaton and Arakan in Lower Burma is *N. goral griseus*. All these are a good deal larger than *N. cranbrooki*, not only in overall measurements but in comparative length of ears and tail. The series of *N. goral griseus* from Yunnan whose external measurements are quoted by G. M. Allen (1940) have tails from 130–150 mm. and ears from 117–140 mm. Thus the red goral appears to be a small and geographically isolated form.

It should be noted that there is no possibility of confusing the red goral with the red serow, *Capricornis sumatraensis rubidus* Blyth, from Lower Burma. The serows are readily distinguishable from gorals not only by their facial glands and their much greater size, but by the very different character of their pelage, which is coarse and stiff and in the case of the red serow is white at the base.

Finally, it is to be hoped that before it is too late, further specimens will be obtained, and further observations made in the field, to throw more light on the status of this interesting species.

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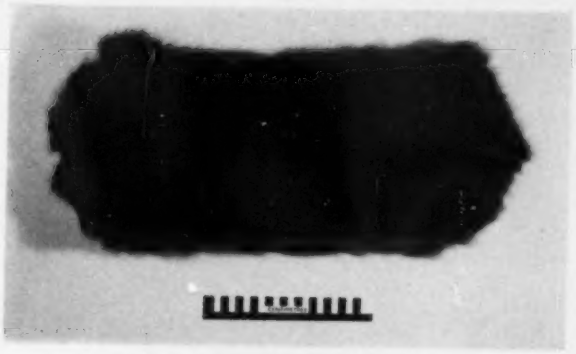
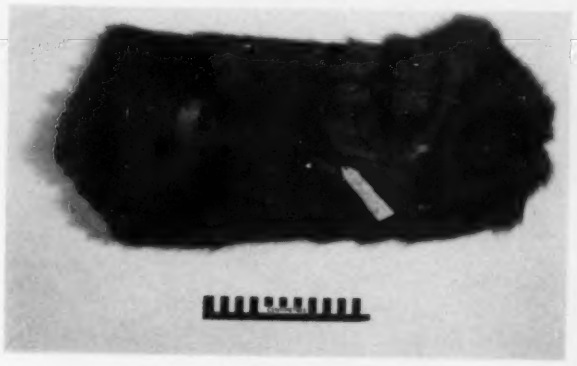
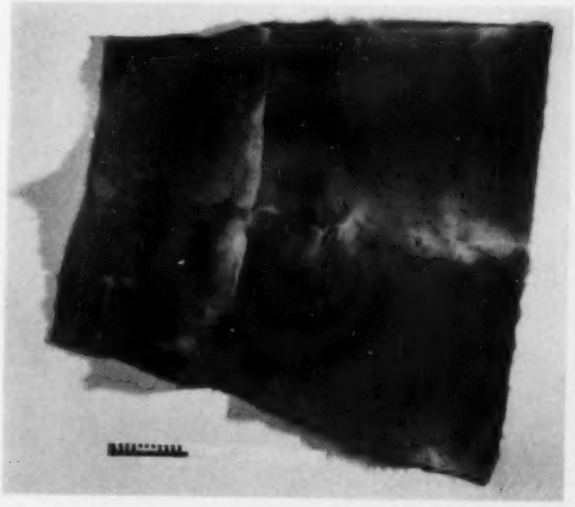
Addendum. While this paper was in press, a very early reference to what is presumably this animal was found. Blyth (1863), after listing Himalayan specimens of *N. goral* in the Museum of the Asiatic Society, Calcutta, remarks "The Asámese" (or Butan?) race is bright rufous. H. *A.* Skin. Presented by Col. Jenkins. Presumably this skin no longer exists, for there has been no further mention of it.



A. *Nemorhaedus cranbrooki*, sp.n. Skin of type
B.M. No. 32.11.1.170 Dorsal view.



B. *Nemorhaedus cranbrooki*, sp.n. Skin of type
B.M. No. 32.11.1.170 Ventral view.



C. *Nemorhaedus cranbrooki*, sp.n. Rug presented by
Mr H. L. Cooper, B.M. No. 60.436

COMPARATIVE STUDIES OF THE NERVE SUPPLY OF THE LARYNX IN EUTHERIAN MAMMALS

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[Accepted 11th October, 1960]

(With 4 plates)

The gross pattern of innervation of the larynx is described in twenty-six species of eutherian mammals. The presence of an internal and recurrent laryngeal nerve is constant in all these species but the communications between the two nerves are variable. Four different types are described. The possible significance of variation in the pattern of innervation is discussed.

CONTENTS

	Page
Introduction	325
Material and methods	325
Observations	326
Discussion	327
Acknowledgments	329
References	329

INTRODUCTION

The classical work on the comparative anatomy of the larynx was published by Negus in 1929. These investigations centred on the evolution, structure and functions of the laryngeal cartilages and muscles but the nerve supply is only briefly mentioned. From a survey of the literature it is apparent that the innervation of the larynx is described only in man and the common laboratory animals, and these accounts are both variable and confusing. It was therefore decided to investigate the nerve supply of the larynx in as many mammalian species as were available.

MATERIAL AND METHODS

The laryngeal nerves and their connections were dissected out on both sides in the larynx of twenty-six species. Details of these and the numbers of specimens are given in Table 1. Dissections were all made on formalin fixed material and the very small species were examined under the dissecting microscope. Particular attention was paid to the existence of any communication between the internal and recurrent laryngeal nerves. In specimens where

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this was present, a piece of it was taken and sectioned at 10μ and stained with haematoxylin and eosin to study the general structure, and with Fielding's silver stain to confirm the presence of nerve fibres. For each species drawings were made and observations were also recorded photographically. (Photographs and diagrams of the twenty-six species have been deposited in the Library of the Zoological Society of London).

TABLE 1

ORDER	GENERA	COMMON NAME	NO. OF SPECIMENS
Insectivora	*Talpa*	Common mole	2
	Erinaceus	Hedgehog	1
Chiroptera	*Pteropus*	Indian fruit-bat	1
	Nyctalus	Noctule bat	1
Lagomorpha	*Lepus (europaeus)*	Hare	1
	Lepus (cuniculus)	Rabbit	7
Primates	*Homo*	Man	14
	Hylobates	Gibbon	2
	Papio	Baboon	2
	Mandrillus	Drill	1
	Cercocebus	Sooty mangabey	1
	Cercopithecus	Diana monkey	1
	Macacus	Rhesus monkey	1
	Saimiri	Squirrel monkey	1
	Hapale	Marmoset	2
Carnivora	*Felis (domestica)*	Cat	5
	Felis (serval)	Serval	1
	Canis (familiaris)	Dog	6
	Lutra	Otter	1
	Phoca	Seal	2
Artiodactyla	*Sus (scrofa)*	Pig	3
	Bos (taurus)	Cow	2
	Ovis (aries)	Sheep	2
	Cephalophus	Duiker	1
	Lama	Llama	1
	Cervus	Deer	1
			63

OBSERVATIONS

All the species examined had both an internal and a recurrent laryngeal nerve but the details of their communications varied considerably. Four different patterns could be distinguished. A representative of each of these types is shown in Plates 1 to 4:—

(A) Supply by the internal and recurrent nerves only with no connection between them. This type of innervation was found in six out of nine primate species and in all the Insectivora, Chiroptera, and Lagomorpha examined. (Plate 1.—Drill).

(B) Supply by the internal and recurrent nerves with a communication, the ramus anastomoticus, between them. This was found in two out of

five species of carnivores, one artiodactyle, man and baboon. (Plate 2.—Human).

(C) Supply by the internal and the recurrent nerves and the ramus anastomoticus and by a median laryngeal nerve. This latter nerve originated either from the superior laryngeal nerve or from the pharyngeal plexus of the vagus and entered the larynx through the superior constrictor muscle. This type was found in all the artiodactyle species examined except the pig but in no other order. (Plate 3.—Llama.)

(D) Supply by the internal and recurrent nerves with the internal nerve making a communication with the pararecurrent nerve (Lemere), which runs parallel with the recurrent nerve to join the vagus. This type is found in three out of five species of Carnivora and the marmoset amongst the Primates. (Plate 4.—Cat).

Table 2 gives the findings for each of the species examined.

A summary is given in Table 3.

TABLE 2

ORDER	GENERA	NO. SPECIMENS	TYPE OF COMMUNICATION			
			A	*B*	*C*	*D*
Insectivora	*Talpa*	2	2	—	—	—
	Erinaceus	1	1	—	—	—
Chiroptera	*Pteropus*	1	1	—	—	—
	Nyctalus	1	1	—	—	—
Lagomorpha	*Lepus (europaeus)*	1	1	—	—	—
	Lepus (cuniculus)	7	7	—	—	—
Primates	*Homo*	14	2	12	—	—
	Hylobates	2	2	—	—	—
	Papio	2	—	2	—	—
	Mandrillus	1	1	—	—	—
	Cercocebus	1	1	—	—	—
	Cercopithecus	1	1	—	—	—
	Macacus	1	1	—	—	—
	Saimiri	1	1	—	—	—
	Hapale	2	1	—	—	1
Carnivora	*Felis (domestica)*	5	—	—	—	5
	Felis (serval)	1	—	1	—	—
	Canis (familiaris)	6	—	6	—	—
	Lutra	1	—	—	—	1
	Phoca	2	—	—	—	2
Artiodactyla	*Sus (scrofa)*	3	—	3	—	—
	Bos (taurus)	2	—	—	2	—
	Ovis (aries)	2	—	—	2	—
	Cephalophus	1	—	—	1	—
	Lama	1	—	—	1	—
	Cervus	1	—	—	1	—
		—	—	—	—	—
		63	23	24	7	9
		—	—	—	—	—

DISCUSSION

In the present series it was only possible to obtain a single specimen of the more rare species. Nevertheless it is apparent that, within the basic

plan, which is remarkably uniform amongst the mammals, there is a great deal of variation, both in shape and proportions of the larynx, and in its pattern of innervation. Variations occur both between species belonging to the same order, and also within a single species. This may explain much of the confusion in the literature as some authors have applied observations drawn from

TABLE 3

ORDER	NO. SPECIES	NO. SPECIMENS	TYPE OF COMMUNICATION			
			A	*B*	*C*	*D*
Insectivora	2	3	3	—	—	—
Chiroptera	2	2	2	—	—	—
Lagomorpha	2	8	8	—	—	—
Primates	9	25	10	14	—	1
Carnivora	5	15	—	7	—	8
Artiodactyla	6	10	—	3	7	—
	—	—	—	—	—	—
	26	63	23	24	7	9
	—	—	—	—	—	—

one species to another. The variation is only apparent if a number of specimens is examined, for example, in man the ramus anastomoticus was absent in two out of the fourteen specimens examined.

The present observations differ in some respects from previous accounts although the innervation of the larynx has been described in relatively few species apart from man and the common laboratory animals. In a series of fourteen dogs Lemere (1932) described a connection between the internal and the pararecurrent nerves in the majority of cases but no actual figures were specified. Vogel (1952) in a series of ten dissections in the same species did not mention the pararecurrent nerve but described an internal-recurrent connection which was confirmed in all six specimens of the present series.

Bourdelle & Bressou (1953), in their monograph on the cat and dog, described an internal-pararecurrent connection but made no distinction between the two species. In the present series however, the internal-pararecurrent connection was found in all five cats examined but an internal-recurrent connection was found in all six dogs.

Howell & Huber (1891) described a communicating branch between the internal and recurrent nerves in the rabbit but in the present series of seven this was not seen in any of the specimens.

The ramus anastomoticus contained nerve fibres in all cases where it occurred but the fact that it was absent in 48 out of 126 (38 per cent) of the nerves examined raises the question of the significance of the ramus anastomoticus and suggests that it is not of fundamental importance in the innervation of the larynx. It is possible that functionally identical fibres might reach the larynx through an alternative route and in the case of secreto-motor fibres, this is known to be so (Lemere, 1932). Another possible function of the ramus anastomoticus which remains to be examined is that it might act as a route through which afferent fibres from proprioceptors in the muscles could pass

into the internal nerve and so reach the central nervous system. Afferent endings in the form of muscle spindles are known to exist in all the laryngeal muscles in man (Lucas Keene, 1957) and in the cricoarytenoideus posterior of the cat (Withington, 1959). Study of both the nerve endings in the muscles and also the measurement of fibres sizes in the laryngeal nerves will be necessary before this problem is solved.

Dilworth (1921), on the basis of dissection in man, suggested that the system of laryngeal nerves was really a plexus. He viewed the ramus anastomoticus as a strand of one of the plexuses of the vagus nerve which during development had become separated off by the thyroid cartilage from the general laryngeal and pharyngeal plexus.

It is difficult to say until a larger series of animals has been studied whether the general complexity of the pattern of innervation is related to phylogeny. The more primitive insectivores, the bats, and most of the monkeys have a simpler type of innervation than man, the Carnivora, and the Artiodactyla. To trace the pre-Eutherian pattern of innervation a series of dissections of the marsupial larynx has been undertaken. Another interesting aspect of this subject is the question of whether the complexity of innervation is in any way related to the range and pitch of the voice of the individual. The larynx is an organ needing a very fine balance of adjustment if any great range of notes or tones is to be produced, and an investigation of voice in different animals may lead to an explanation of the functional significance of these observations.

ACKNOWLEDGMENTS

It is a pleasure to record our thanks to the Council of the Royal Free Hospital School of Medicine for the award of a Junior Research Fellowship to J.L.S. and to the Medical Research Council for a grant towards the cost of expenses.

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We also owe thanks to Miss F. M. Ellis, Mrs P. M. Thomas, and Mr R. T. Fletcher for photography and to Mr W. Matthews and Miss I. Johnson for technical assistance with histological preparations.

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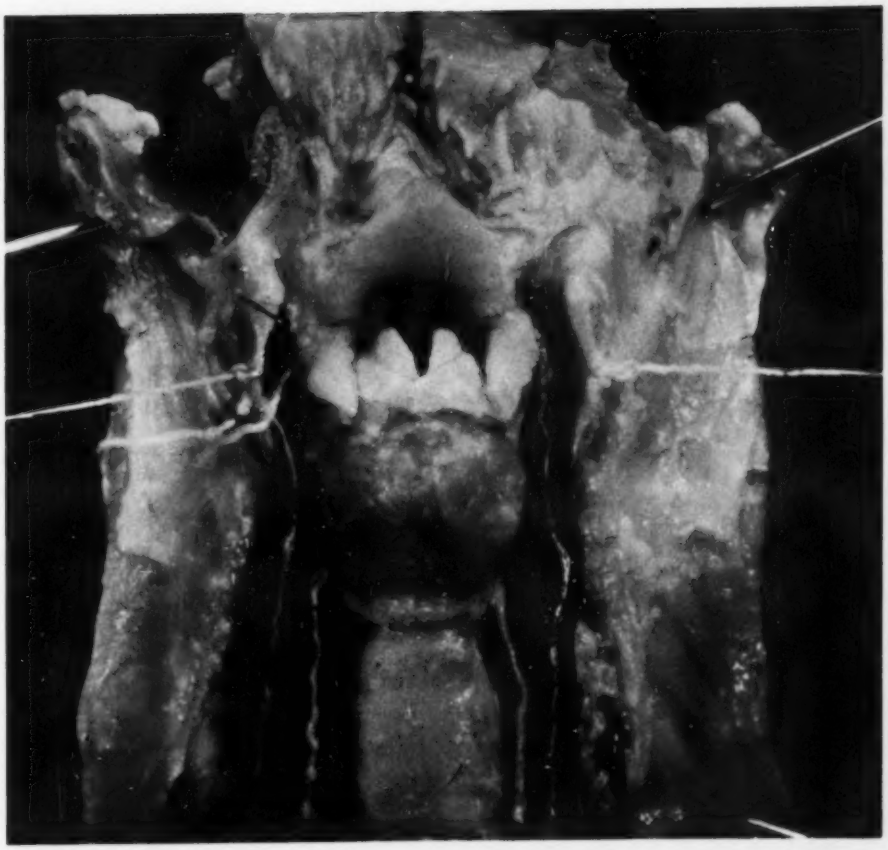
Dissection of the larynx from the posterior surface.
Type A pattern of innervation—Drill (*Mandrillus*).

cms.

Dissection of the larynx from the posterior surface.
Type B pattern of innervation—Human (*Homo*).

Dissection of the larynx from the posterior surface.
Type C pattern of innervation—Llama (*Lama*).



Dissection of the larynx from the posterior surface.
Type D pattern of innervation—Cat (*Felis*).

A NEW GENUS AND SPECIES OF LEECH PARASITIC ON *DREPANOPSETTA PLATESSOIDES* (MALM) THE LONG ROUGH DAB.

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[Accepted 11th October, 1960]

(With 3 figures in the text)

This new piscicolid leech (subfamily Ichthyobdellinae) was found attached to the branchiae and inner surface of the branchial chamber of *Drepanopsetta platessoides*, trawled off Iceland. It has a fairly extensive coelomic system with no clear separation into dorsal, ventral and segmental lacunae. Its posterior crop caeca are separate and there are only eighteen separate ganglia between the anterior and posterior ganglionic masses.

CONTENTS

	Page
Introduction	331
Generic characters	332
External character	332
Internal anatomy	333
Body wall	333
Gland cells	334
Gut	335
Nervous system	336
Coelomic system and blood vessels	337
Excretory system	337
Reproductive system	339
Comparison with related forms	339
Acknowledgment	341
References	341

INTRODUCTION

Four leeches were found on three separate specimens of *Drepanopsetta platessoides* (Malm.), the long rough dab, in a sample of about four hundred and ten of these fishes trawled off the north-west and the south-east coasts of Iceland. The leeches were attached to the gills and branchial chamber of the hosts. They were found more or less curled up and contracted, some time after preservation of the fishes in formalin.

Nevertheless, one complete series of more or less transverse sections was prepared, cut at 9μ and stained in Ehrlich's haematoxylin and eosin. Another specimen was compressed slightly and stained in borax carmine. The sections showed that this species has in the testicular region a fairly extensive coelomic system, not divided into transverse segmental lacunae, and also has the posterior

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pair of crop caeca (the " blind sacs ") completely separate from one another. This combination of characters, which have often been regarded as primitive, has not hitherto been described from the subfamily Ichthyobdellinae, to which the leech clearly belongs. It is, therefore, proposed that this should be the type for a new genus. The systematic position of this species and the differences which distinguish it from related forms are discussed later.

GENERIC CHARACTERS

ARCTOBDELLA gen. n.

Skin smooth without dorsal papillae, pulsatory vesicles, gills or other external metameric markings, except for some indistinct annulation. No eyes distinguishable in preserved specimens. Anterior sucker (in contraction) very small. Posterior sucker large, a little narrower than maximum body width. Posterior crop caeca separate. Coelomic system ill-defined but extensive, with an indistinct dorsal lacuna and a spacious ventral lacuna. In the testicular region, the ventral lacuna communicates more or less continuously with the dorsal lacuna, there being no restriction of the coelom to form segmental or accessory communications. About four pairs of testes. Vesiculae seminales present.

Type species—**Arctobdella branchiarum** sp. n.—Deposited in the Dept. of Zoology, Univ. College of Swansea, Registration No.—H.3.

Host—*Drepanopsetta platessoides* (Malm.)

EXTERNAL CHARACTERS

(Fig. 1)

The lengths of the preserved specimens lie between 9·7 and 12·6 mm. The body is flattened dorso-ventrally and is widest about mid-abdomen. The preclitellar region is only a little wider posteriorly than anteriorly. Its maximum width is about one third of that of the body and its length nearly one twelfth of the total body length. The clitellum is hardly recognisable externally. The contracted anterior sucker is narrower than the smallest preclitellar width and measures 0·25—0·35 mm. in diameter. The posterior sucker is large, discoid and 1·3—1·7 mm. in diameter. It is about five times the size of the anterior sucker but a little narrower than the greatest body width.

No eyes are discernible on the head or on the adjoining annuli of the " neck " in the preserved specimens.

The skin is smooth. The number of annuli per segment would appear to be about three, but these are often subdivided and the annulation is therefore irregular and indistinct. The preserved specimens are pale yellowish grey, with the margins of the body and the posterior sucker somewhat translucent. Dermal cells, which appear as brownish flecks, as well as larger, paler clitellar gland cells, can be seen through the skin. No pigment spots are discernible on the body and the posterior sucker is without eye spots or other markings. The clitellum is more opaque than the rest of the body. The male genital aperture is seen as a small, oval depression mid-ventrally about the middle of the clitellum, but the female pore is not easily discernible externally.

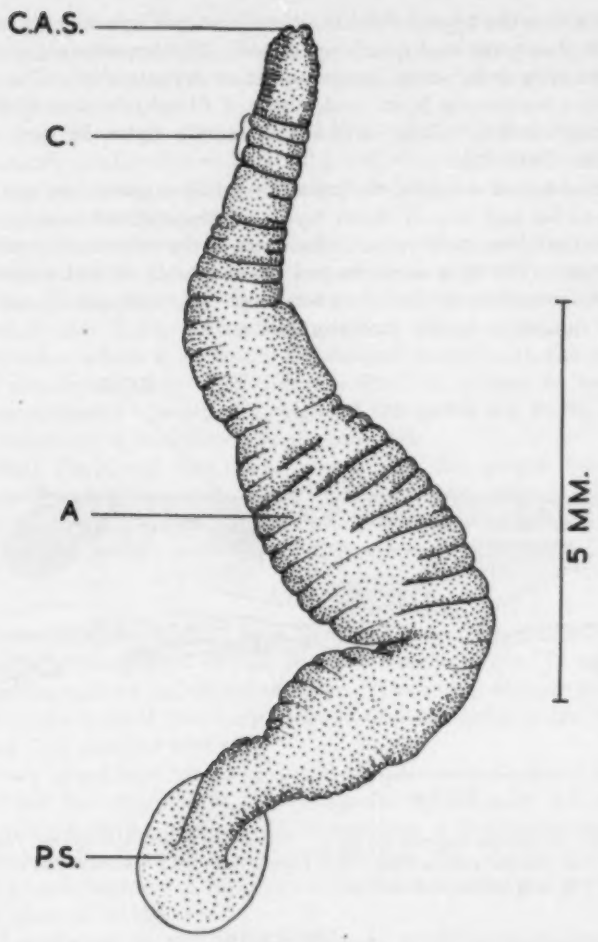

Fig. 1—*Arctobdella branchiarum* sp. n. Dorsal view of a preserved specimen. A, abdomen ; C, clitellum ; CAS, contracted anterior sucker ; PS, posterior sucker.

INTERNAL ANATOMY

The numbering of segments is explained in the section on the nervous system (p. 336).

Body wall (Fig. 2)

The cuticle is thin, 1—3μ in thickness and smooth. The epidermis consists of a row of cells which are somewhat elongated radially to the body. They are 10—17μ long by 5—12μ wide, with their inner walls rounded. The dermis is thinnest dorsally (20—25μ) and thickest laterally (57—60μ). The dermal cells lying dorsally and ventrally are large (50—112μ by 15—37μ) and ovoid,

practically filling the hypodermis, but those situated laterally are much smaller (35—40μ in diameter) and nearly spherical. The hypodermis also includes a few fibre forming cells, which are more numerous laterally. The dermal cells do not form a continuous layer, as do those of *Platybdella anarrhichae* (Diesing) (Leigh-Sharpe, 1916). Their nuclei are usually flattened and no pigment granules are discernible.

The musculature consists of a layer of circular muscles, one or two layers of oblique muscles and two or three layers of longitudinal muscles. The total width of these layers is 30—67μ. Two sets of dorso-ventral muscles could be distinguished. The first series occurs on each side of and close to the gut, whereas the second series lies more laterally. Dorso-ventral muscle fibres are extremely numerous on the posterior sucker.

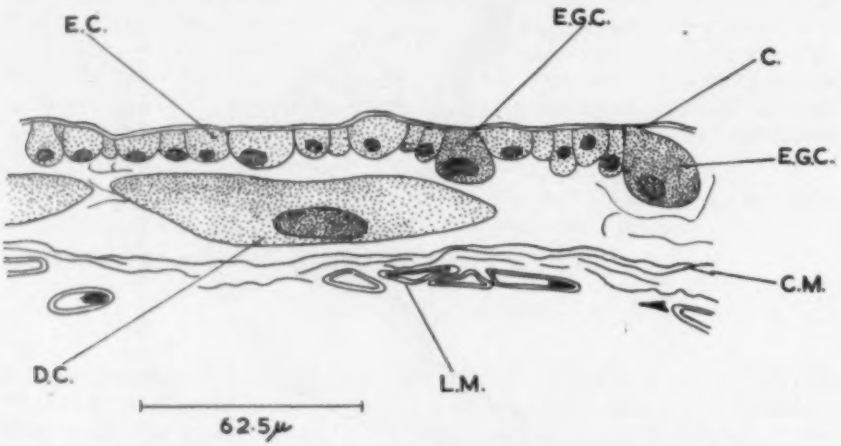

Fig. 2—*Arctobdella branchiarum* sp. n. Transverse section through the body wall.
C, cuticle ; CM, circular muscle fibre ; DC, dermal cell ; EC, epidermal cell ; EGC, epidermal gland cell ; LM, longitudinal muscle fibre.

Gland cells

(1) *Epidermal gland cells.* Numerous gland cells lie in the epidermis and project into the hypodermis. These occur irregularly over the whole surface of the body. Each gland cell is usually globular with a wide opening to the exterior. A few flask-shaped gland cells also occur with narrow ducts opening to the exterior between the epidermal cells. Their nuclei are oval to spherical, generally situated in the middle of the gland cells. The gland cells are 22—45μ in diameter.

(2) *Sucker gland cells* are seen only on the anterior sucker. They are flask-shaped, 17—25μ in diameter, with long narrow ducts opening to the exterior on the outer margin of the sucker. Although they are not numerous, they occur round the whole margin.

(3) *Preclitellar gland cells* are ovoid, 25—67μ in great diameter, and lie centraliy to the dermal musculature. Their nuclei are large, ovoid and 15—37μ

in maximum diameter. These cells are without large secretion spaces, their coarsely granular secretion being distributed throughout the general cytoplasm. The secretion, however, appears to be especially concentrated towards the origins of the ducts.

The openings of the preclitellar gland ducts are well distributed dorsally and dorso-laterally on the first and second preclitellar segments. On the third preclitellar segment, they are well distributed dorsally, but concentrated in two narrow groups dorsolaterally.

(4) *Clitellar gland cells* are more numerous and their distribution extends from behind the clitellum to the base of the posterior sucker. Two types of these cells could be distinguished by the nature of their secretion. Those of the first type, which are 57–87μ across their greatest diameter, possess a coarsely granular secretion which is generally distributed throughout the cytoplasm. The cells of the second type, which measure 60–112μ, possess an homogenous secretion, concentrated towards the origin of the gland cell ducts. There is no large secretion space in either type of gland cell.

The clitellar gland cell ducts are arranged for the greater part of their length in eight bundles, two dorsal, two ventral and two on each side (Fig. 3). Ducts carrying the two types of clitellar secretion lie together in a given bundle and open separately over the general surface of the clitellum.

Gut.

The proboscis is short, with its base (posterior end) at about the level of the second preclitellar ganglion. It has the usual structure, with trifid lumen and circular, longitudinal and radial muscles. The radial muscles are sparsely developed, but the layer of circular muscles is 12·5–25μ thick at the base of the proboscis and 7·5μ thick at its tip.

The salivary gland cells are large, ovoid to spherical and 80–115μ in great diameter. They lie centrally to the preclitellar gland cells and are found throughout the preclitellar region. Their secretion is finely granular. Their ducts enter the proboscis at its base and lie between the circular and the outer longitudinal muscle layers. A few open along the length of the proboscis but the majority open at its tip.

The gut lumen expands immediately posterior to the proboscis but becomes narrower between the oesophageal glands. There is a pair of these glands extending from segment 10 to the middle of segment 12. Each is a large, highly distended pouch, with a layer of circular muscles and a rather indistinct layer of flattened epithelium, which is not folded. The glands appear to be filled with a highly staining secretion. Their ducts are narrow and oval, 12 by 5μ in diameter and open into the oesophagus just anterior to the second clitellar ganglion.

A few ingested red blood corpuscles could be seen in the oesophagus. The region immediately following, constituting the crop and its caeca, was filled with red blood corpuscles.

The crop is thin-walled, with weak muscle fibres occasionally seen outside its walls. Its epithelium is of flattened cells and intensely folded. It gives off a pair of lateral caeca in each segment of the testicular region except the first.

These are progessively larger posteriorly. The crop is provided with a sphincter anterior to the second, third and fourth pairs of crop caeca, there being no sphincter anterior to the first pair. The posterior crop caeca arise between segments 18 and 19 and open separately into the crop. The crop is provided with a sphincter anterior to its connections with these caeca. These caeca lie more or less ventro-laterally to the intestine. Their epithelium consists of a single layer of flat cells and is not folded. They extend to about segment 24 and remain completely separate throughout their length, although their lateral walls are often in contact with one another.

The intestine is large, with a layer of circular muscles, and is lined by a layer of flat, ciliated epithelial cells. This ciliated epithelium is deeply folded and occurs throughout the whole length of the intestine as well as in the intestinal pouches. Only two pairs of symmetrically arranged lateral intestinal pouches could be observed. These occur in the first and second segments of the region of the posterior crop caeca.

The rectum is short, with deeply folded walls. It becomes dorsal to open by the anus at the base of the posterior sucker.

Nervous system

Most leeches have twenty-one separate body ganglia, but in *Arctobdella branchiarum*, as in *Abranchus blennii* Knight-Jones (1940) and *Sanguinothus pinnarum* de Silva & Burdon-Jones (1960), there are only eighteen such ganglia.

The ganglionic capsules composing the suboesophageal mass were therefore studied in order to determine whether any fusion of the ganglia had occurred anteriorly. The number of ganglionic capsules to be seen in single transverse sections, throughout most of the length of the suboesophageal mass of this species, ranges, as in leeches generally, from five to nine. In the posterior part, however, for about one-sixth of the total length of the suboesophageal mass, the number of capsules remains at three. It therefore seems likely that this region represents a normal segmental ganglion completely fused with the suboesophageal mass, which would, therefore, correspond with the first preclitellar ganglion of other leeches. This conclusion is confirmed by the positions of the clitellum, the genital pores and the origin of the posterior crop caeca. Assuming that the first preclitellar ganglion, corresponding to segment 7, has fused anteriorly thus, these structures would lie in the following segments, agreeing with their position in other leeches :—

Clitellum	segments 10 to 12.
Male genital pore	posterior part of segment 11.
Female genital pore	anterior part of segment 12.
Opening of posterior crop caeca	between segments 18 and 19.

The second preclitellar ganglion arises almost immediately posterior to the first, the thickness of the commissure separating the two ganglia being less than 18μ. The third preclitellar ganglion lies at a distance of about 36μ posterior to the second.

The pattern of fusion posteriorly remains difficult to decide. The last of the eighteen separate body ganglia is slightly larger than the others, measuring

189μ in length. The number of ganglionic capsules seen within it in one plane varies from four to six, though in the posterior half it remains at three. The preceding six ganglia measure 144μ, 108μ, 162μ, 135μ, 162μ, and 180μ in length respectively. The last of these ganglia contains the usual number of six capsules, of which no more than three appear in a given transverse section. The ganglionic capsules composing the ganglionic mass of the posterior sucker were studied and their number did not appear to exceed the usual forty-two. It therefore seems likely that the last separate body ganglion represents a ganglionic mass resulting from fusion of the three anal ganglia of other leeches (segments 25 to 27). It would appear that the pattern of fusion of ganglia is the same as in *Abranchus blennii* and *Sanguinothus* (de Silva & Burdon-Jones, 1960).

Coelomic system and blood vessels (Fig. 3)

The dorsal blood vessel extends from the first preclitellar segment to the intestine where it forms an intestinal blood sinus. It fills the dorsal lacuna, which is only occasionally discernible as a number of minute cavities adjoining the dorsal blood vessel.

The lacunae are not bounded by well-defined walls and it is, therefore, difficult to discern their exact limits. The ventral lacuna is generally spacious. It extends from the base of the proboscis to the base of the posterior sucker. It contains within it the nerve cord throughout its length, the ovaries and the ventral blood vessel whenever the latter can be seen. What might be regarded as lateral lacunae could be distinguished with difficulty as irregular, thick-walled, lateral cavities, but these were seen only in the first and second segments of the testicular region.

In the testicular region, opposite each nerve ganglion, the ventral lacuna extends laterally and becomes somewhat widened near the midline. Its lateral extensions, however, are narrow, penetrating between the groups of clitellar bundles. A narrow, ill-defined space surrounds the crop and is traversed by irregularly arranged trabecular tissue and a few dorso-ventral muscle fibres. This space communicates with the ventral lacuna. It can be seen both segmentally and inter-segmentally throughout the testicular region. Since it forms a communication between the ventral lacuna and the small irregular dorsal lacuna, it appears to correspond both to the main segmental communications and to the accessory (inter-segmental) communications which are found in the testicular region of most Piscicolidae.

The testes appear somewhat shrunken and lie in spacious cavities, the testicular lacunae. Each testicular lacuna communicates with the dorsal and ventral lacunae, though it is incompletely separated from the ventral lacuna by fibrous tissue resembling a disintegrated membrane. No structures comparable with hypodermal pulsatile vesicles or contractile marginal lacunae are to be seen.

Excretory system

A pair of nephridial tubules could be seen laterally, closely anterior to the testes in the first and second segments of the testicular region. No other tubules could be distinguished in the series of sections studied.

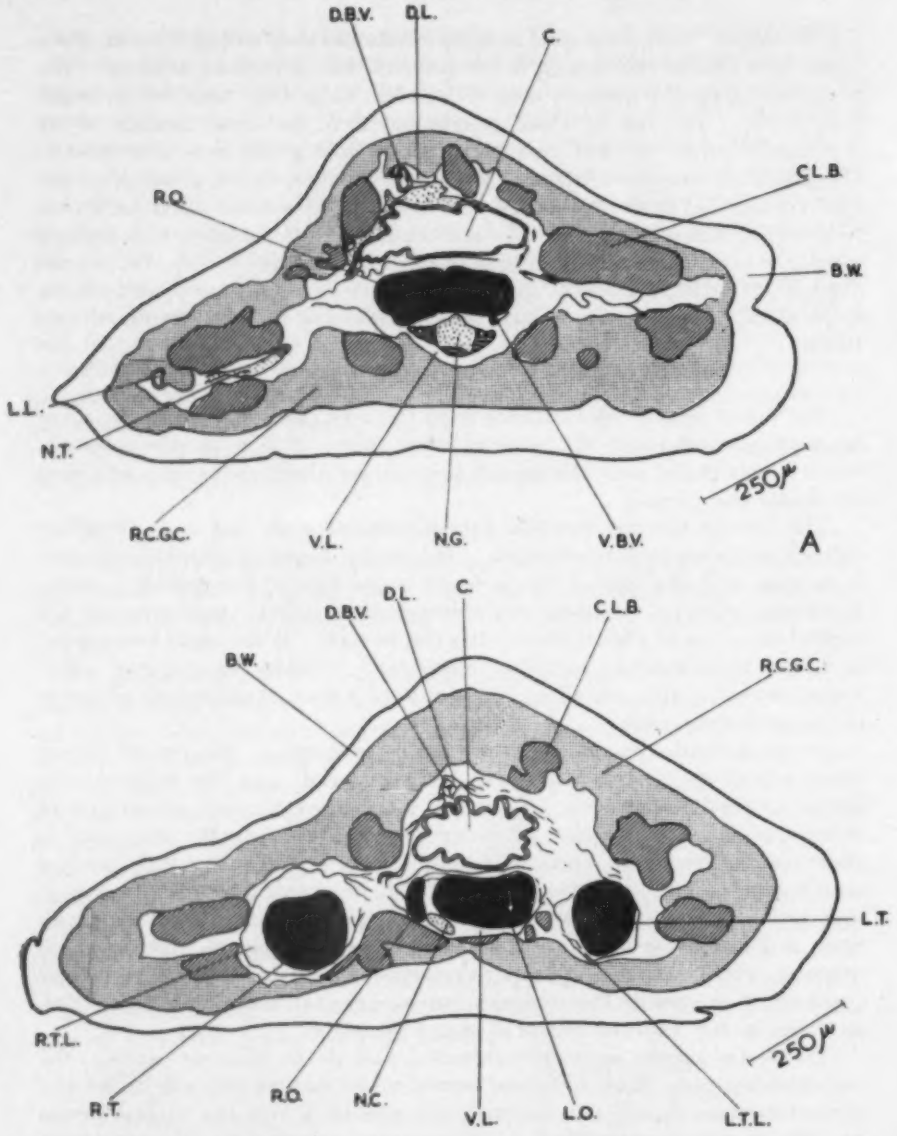

Fig. 3.—*Arctobdella branchiarum* sp. n. A, transverse section of body through 2nd ganglion of testicular region ; B, transverse section between 3rd and 4th ganglia of testicular region. BW, body wall ; C, crop ; CLB, bundles of clitellar gland ducts (cross-hatched areas); DBV, dorsal blood vessel ; DL, dorsal lacuna ; LL, lateral lacuna ; LO, left ovary ; LT, left testis ; LTL, left testicular lacuna ; NC, nerve cord ; NG, nerve ganglion ; NT, nephridial tubule ; RCGC, region of clitellar gland cells (uniformly dotted area) ; RO, right ovary ; RT, right testis ; RTL, right testicular lacuna ; VBV, ventral blood vessel ; VL, ventral lacuna.

Reproductive system

There are three pairs of testes and the left member of a fourth pair, arranged inter-segmentally in segments 14/15 to 17/18 of the specimen sectioned. The vasa deferentia are at first lateral and then dorso-lateral in position. They widen to form the vesiculae seminales at about the posterior limit of the first testicular segment. The vesiculae seminales are dorso-lateral in position and highly sinuous. They are surrounded by a layer of circular muscles and lined by a layer of flat epithelial cells. They lead into the ducti ejaculatorii in the anterior part of the first testicular segment. The terminal parts of these begin in the anterior part of the third clitellar segment and open into a common atrium in the middle part of the second clitellar segment.

The ducti ejaculatorii and the atrium have the structure found in most Piscicolidae. Numerous elongated glandular cells radiate into the lumen of the terminal parts of the ducti ejaculatorii and the atrium. The atrium opens into a bursa, which has thick folded walls, with two to three layers of circular muscle fibres and a lining layer of flattened epithelial cells which have large nuclei. The bursa opens to the exterior in segment 11.

The ovaries are long, tubular structures extending from segments 13 to 18 and lying ventrally to the crop. Anteriorly to segment 14, they lie outside the ventral lacuna, but posteriorly they lie within this lacuna. *Branchellion*, *Pontobdella* and *Trulliobdella* also have their ovaries lying within the ventral lacuna. The oviducts run anteriorly, ventro-laterally at first and then mid-ventrally, to open into the vagina about the middle part of segment 12. The vagina is thick-walled and lined with radially arranged, somewhat elongated cells with large nuclei. It opens to the exterior through the female genital pore which lies mid-ventrally on the anterior part of segment 12.

A tissue somewhat similar to the conducting tissue of *Crangonobdella* (Selensky, 1915, Pl. VI, fig. 13) lies between the oviducts and the mid-ventral dermal musculature. There is no distinct copulatory area, but the dermis close to the female genital pore is exceptionally thick (about 75μ), with numerous flask-shaped epidermal gland cells and muscular fibrillae. The overlying cuticle is flat and not folded. This area is very narrow and extends for a very short distance posteriorly to the female genital pore.

COMPARISON WITH RELATED FORMS

Arctobdella branchiarum is a member of the subfamily Ichthyobdellinae, since it possesses a smooth skin without pulsatile vesicles, gills, papillae or other external protuberances. Its dermis contains large dermal cells filling up the hypodermis (Fig. 2) as in *Abranchus*, *Ottoniobdella* and *Sanguinothus*, but not *Platybdella*. It possesses a fairly extensive coelomic system, comprising a large ventral lacuna which is more or less continuous with what may be regarded as ill-defined dorsal, lateral and segmental lacunae. The nerve cord and the ventral blood vessel lie within the ventral lacuna.

Johansson (1898) classified the coelomic system of Piscicolidae into three main types, his scheme being slightly modified subsequently by Selensky (1915 and 1931). These three types, according to Selensky, are :

(1) with all the main parts well developed and usually possessing one or two pairs of pulsatile vesicles to a segment, e.g. *Johanssonia*, *Callobdella*, *Piscicola*, *Scorpaenobdella* (= *Trachelobdella*), *Oxytonostoma*, *Pontobdella* (the vesicles in *Pontobdella* are exceedingly small), etc. ;

(2) with a more or less reduced coelomic system, as in *Abranchus*, *Platybdella* and *Crangonobdella* ; and

(3) with a comparatively spacious coelom, as in *Ozobranchus*, which has wide lacunae connecting freely with one another, so that there is no well marked division into main (segmental) and accessory (inter-segmental) transverse communications.

The coelomic system of *Arctobdella branchiarum* falls into the third type. The coelom in the testicular region forms a common cavity, broken up only by trabecular fibres. It surrounds the gut and also contains within it the testes. This condition is similar to that found typically in *Ozobranchus*, though in *O. margoi* there is an incomplete division into main and accessory communications (Selensky, 1915). Most workers on piscicolid leeches agree that the subfamily Branchiobdellinae is primitive and that the reduced coelomic system of the Ichthyobdellinae is a result of a greater development of body parenchyma. The only member of the Ichthyobdellinae previously described as possessing a spacious coelom is "*Ichthyobdella*" *platycephali* (Ingram, 1957). It is of interest, therefore, to find another leech which belongs to this subfamily yet possesses such an apparently primitive character.

The posterior crop caeca of *Arctobdella branchiarum* are completely separate from one another. Johansson (1898) classified these caeca of piscicolid leeches according to the extent of their fusion with one another into three types, namely:

(1) completely separated caeca as in *Abranchus* ;

(2) completely fused, single caecum as in *Pontobdella* ; and

(3) intermediate forms.

Leuckart considered the single caecum primitive but both Johansson (1898) and Selensky (1915) considered the paired condition primitive. Johansson (1898) considered the single caecum to be useful in offering greater food storage space to those leeches which leave their hosts temporarily, for instance during the breeding season, but this view was not acceptable to Selensky (1915). The caeca of *Arctobdella branchiarum* place this species into type 1 of Johansson, resembling *Abranchus*, *Ottoniobdella* and *Sanguinothus*. "*Ichthyobdella*" *platycephali* possesses completely fused caeca with two fenestrae (type 3 of Johansson).

The choice of taxonomic characters sufficiently important to characterise new piscicolid genera is still arbitary. Some genera have been characterised by single characters visible externally such as the lateral fin-like expansions of the body of *Pterobdella* (Kaburaki, 1921) or the grouped eye spots of *Janusion* Leigh-Sharpe (1933). The internal anatomy of most of these genera is not yet fully described, so their relationships to other forms cannot be assessed. Johansson and Selensky considered annulation, the nature of the coelomic system and the extent of fusion of the posterior crop caeca as of great systematic value. It is now clear that these characters vary to a great extent independently of one another, and it is therefore less likely that they are of great importance systematically. Nevertheless, it seems impossible, in the present state of our knowledge, to point to any other system as being obviously more

important. The present species is as isolated as any, so the establishment of a new genus for it seems well justified.

Apart from the coelomic system, there is no very marked resemblance between *Arctobdella branchiarum* and "*Ichtyhobdella*" *platycephali*. *A. branchiarum* differs from the latter species in having a more flattened body, four (?) pairs of testes, spermatophore gland cells within the terminal parts of the ducti ejaculatorii, the ventral blood vessel within the ventral lacuna and completely separate posterior crop caeca.

Ingram (1957) remarked that "*Ichthyobdella*" *platycephali* resembled *Johanssonia abditovesiculata* Moore (1952). The inclusion of *J. abditovesiculata* by Moore in the genus *Johanssonia* Selensky (1914 a & b) was only provisional. Although this species resembles *J. koalensis* Selensky in the nature of the annulation of the mid-body somites, the extent of fusion of the posterior crop caeca, absence of eyes and the presence of the ventral blood vessel outside the ventral lacuna, yet its inclusion in this genus appears to rest on the interpretation as vesicles of ten pairs of clear white spots metamerically arranged in segments 8 to 22 (inclusive) and the "marginal lacunar processes" of the main segmental lacunae. The coelomic system of *J. abditovesiculata* has not been fully described and "*Ichthyobdella*" *platycephali* has been included by Ingram in an already rejected genus.

ACKNOWLEDGMENT

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A NEW GENUS AND SPECIES OF LEECH PARASITIC ON THE FISH *COTTUS BUBALIS*

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(With 1 plate and 5 figures in the text)

Five specimens of a new leech were found attached to fin-rays of the dorsal and caudal fins of *Cottus bubalis*. The posterior sucker is folded laterally so that it can partially enclose a fin-ray. These small, smooth-skinned leeches resemble *Platybdella* in some respects, *Abranchus* in others. The coelomic system consists only of dorsal and ventral lacunae, the posterior crop caeca are separate and the dermis is narrow. There are only eighteen separate ganglia between the anterior and posterior ganglionic masses.

CONTENTS

	Page
Introduction	343
Generic characters	344
Habits	344
External characters	345
Internal anatomy	347
Body wall	347
Glands	348
Gut	349
Nervous system	350
Coelomic system and blood vessels	352
Excretory system	352
Reproductive system	352
Comparison with related species	354
Acknowledgments	356
References	356

INTRODUCTION

In March, 1948, five leeches were found on a single specimen of *Cottus bubalis* Euphrasen, which was about 7 cm. long and was collected at low water spring tides near Rhoscolyn, on the south coast of Holyhead Island, Anglesey. Recently Mr P. J. Miller of the Marine Biology Station, Port Erin, found two more leeches of the same species, each on a *C. bubalis*. All these leeches were attached to fin-rays (one of a pectoral fin, the others of postero-dorsal and caudal fins) the posterior sucker being folded laterally to clamp round a fin-ray. There seems to be no previous description of a piscicolid leech modified for attachment to fin-rays. So far as we are aware, only eleven species of marine

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leeches have previously been recorded from British waters, viz. *Pontobdella muricata* (L.), *Branchellion torpedinis* Savigny, *Callobdella lophii* Beneden and Hesse (Harding, 1910), *Ganymedebdella cratere* Leigh-Sharpe (1915), *Callobdella nodulifera* (Malm), *Platybdella anarrhichae* (Diesing) (Leigh-Sharpe, 1916), *Janusion scorpii* (Malm), *Branchellion boreale* Leigh-Sharpe (1933), *Abranchus blennii* Knight-Jones (1940), *Abranchus microstomus* Johansson and *Hemibdella soleae* Beneden and Hesse (Knight-Jones, 1960).

The leeches from *Cottus bubalis* were studied alive and then fixed in sea water Bouin. During fixation, one was slightly compressed and subsequently gower-stained and mounted in balsam (Plate 1). A complete series of transverse sections, 10μ thick, was prepared from another specimen. These were stained in Ehrlich's haematoxylin and eosin.

The sections showed that this species has a very reduced coelomic system like that of *Platybdella anarrhichae* (Diesing), which was described from British waters by Leigh-Sharpe (1916). In other respects, however, notably in having the long posterior pair of crop caeca (the " blind sacs ") completely separate from one another, it is quite different from *Platybdella* and resembles *Abranchus* Johnasson (1896). Its nervous system is like that in *Abranchus blennii* Knight-Jones (1940), showing a pattern of fusion between ganglia which has not been described from other leeches.

If we are to be consistent with the view of Johansson (1898) and Selensky (1915, 1931), who were the chief authorities on the Piscicolidae, we must give great weight to the resemblance of the coelomic system to that of *Platybdella*. But since this is the only important respect in which this form resembles *Platybdella*, it should clearly be placed in a new genus, for which we propose the name *Sanguinothus* (nothus—a mongrel) to denote its intermediate position between *Platybdella* and *Abranchus*.

GENERIC CHARACTERS

SANGUINOTHUS gen. n.

Skin smooth without pulsatory vesicles, dorsal papillae, gills or other external prominences. Mid-body segments triannulate, or further subdivided into six annuli. Suckers well developed. Dermis narrow and filled with large unpigmented dermal cells. Posterior crop caeca separate. Coelomic system greatly reduced, leaving only dorsal and ventral median lacunae.

Type species—*Sanguinothus pinnarum* sp. n.—Deposited in the Dept. of Zoology Univ. College of Swansea, Registration No.—H. 2.

Host—*Cottus bubalis* Euphrasen.

SANGUINOTHUS PINNARUM sp. n.

Habits

Sanguinothus pinnarum remains contracted whilst its host is swimming and is somewhat flattened whilst resting (Fig. 1A and B). If put into a dish, it will walk about in the manner characteristic of leeches, but generally rests, attached by the posterior sucker. It was not seen to swim. The shadow of a hand placed between the dish and the window induces it to extend its body

to about 1·8 times its resting length (Fig. 1C) and swing it about in searching movements, with the anterior sucker widely expanded. A similar response has been observed in several Piscicolidae separated from their hosts (Herter, 1935). Presumably it helps them to find new hosts.

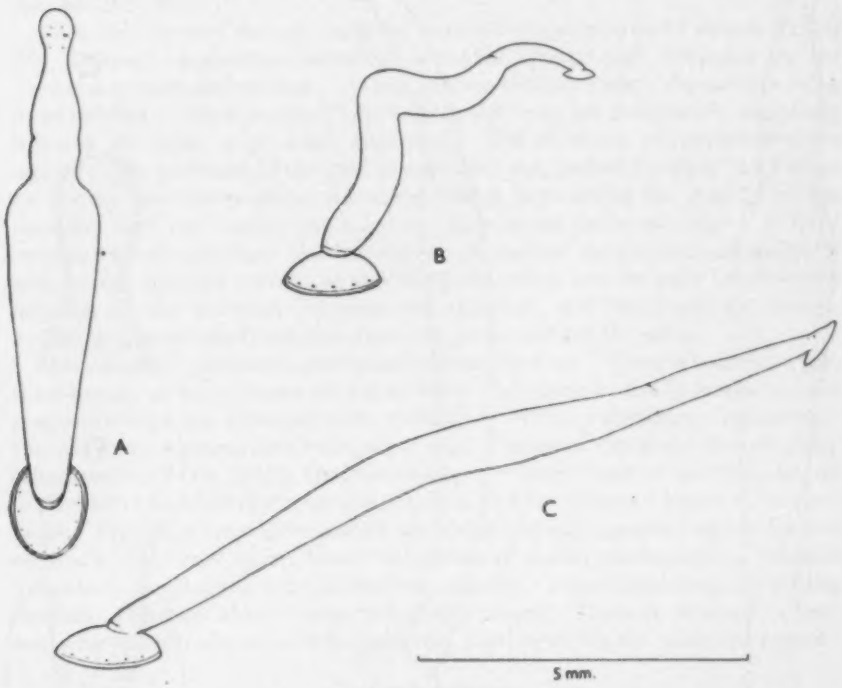

Fig. 1—*Sanguinothus pinnarum* sp. n. A, dorsal view ; B, from right side, showing abdominal flexure sometimes adopted when resting ; C, extended.

External characters

The length when fixed or resting is about 8–12 mm. The body is widest in the region immediately posterior to the clitellar constriction (Fig. 2A). When contracted, the maximum width is about 2 mm. When fully extended, it is about 0·8 mm. The body is usually slightly flattened dorso-ventrally, but is sometimes nearly cylindrical. When most flattened, the width is nearly twice the depth. The preclitellar region is about a fifth of the total body length. Its maximum width is nearly two-thirds that of the body. It is widest posteriorly and becomes gradually narrower towards the anterior sucker.

The anterior sucker, when contracted in the resting condition, is cup shaped and only slightly broader than the narrowest portion of the preclitellar region. When expanded, it is discoid and almost twice its resting breadth (Fig. 2C). There are three pairs of eyes, as in most piscicolid leeches, two

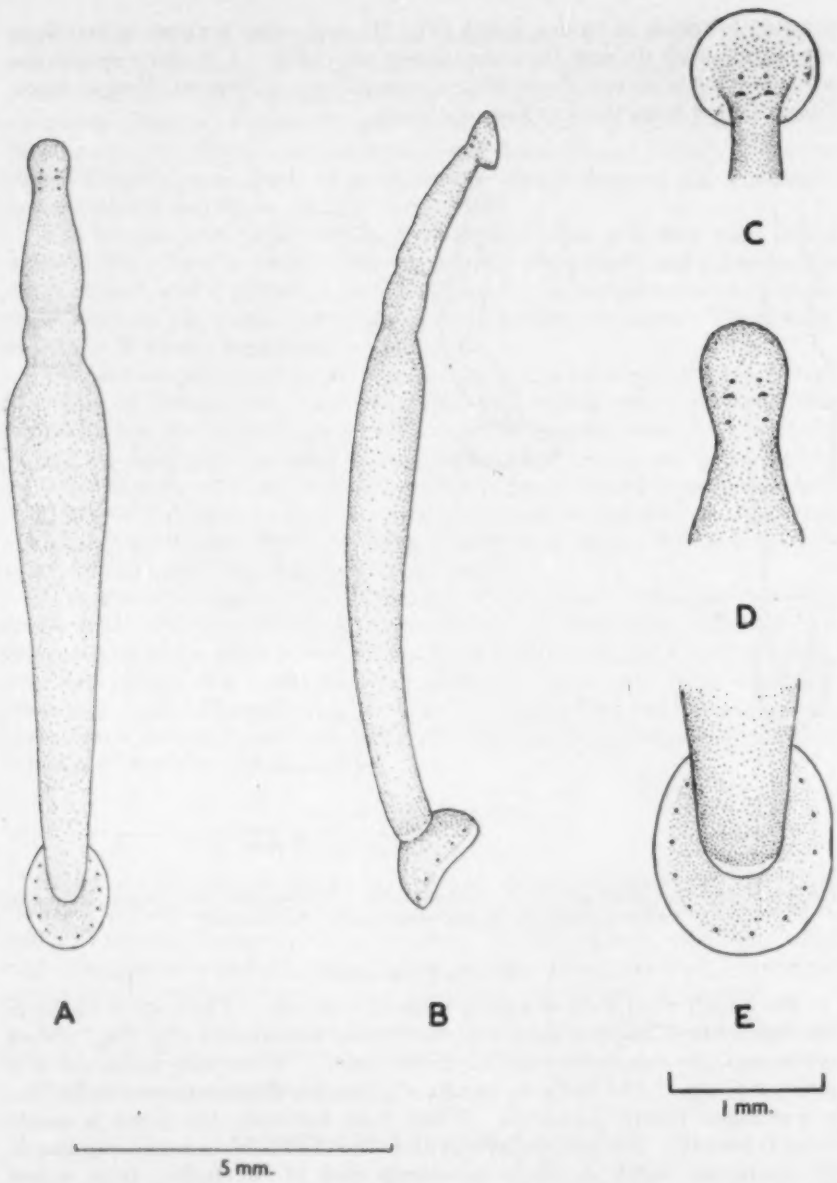

Fig. 2—*Sanguinothus pinnarum* sp. n. A, dorsal view ; B, lateral view ; C, head with sucker dilated ; D, head with sucker contracted ; E, posterior end, dorsal view.

pairs on the head and a pair on the adjoining " neck " (Fig. 2D), but there are no other pigment spots on the anterior sucker.

The posterior sucker is oval, 1·75–2·0 mm. long and 1·2–1·5 mm. broad. It continues to remain somewhat laterally compressed after removal from the fin-ray to which it has been attached. It is about twice the width of the resting anterior sucker, but is not so wide as the body at its widest. There is a ring of about fourteen small black spots around the posterior sucker, near its margin (Fig. 2E).

The skin is very smooth, with no annulations permanently visible during life, although numerous transverse wrinkles appear and disappear as the animal contracts and extends. It is a uniform reddish brown, the suckers being more reddish. The margins of the suckers and body are translucent, especially towards the outer edge when expanded. The clitellum is somewhat more opaque. The positions of the genital apertures are marked by small paler areas on the mid-ventral surface, the male genital pore about the middle of the clitellum and the female genital pore close to its posterior edge. A faint longitudinal streak joins the two areas. A narrow transverse pale stripe is seen at the anterior margin of the clitellum and a narrow pale band occurs dorsally at the junction between the abdomen and the posterior sucker, including a particularly conspicuous pale patch around the anus.

In preserved specimens annulation is more distinct. There are about sixty-three annuli, of which there are about forty-four posterior to the female genital pore and forty-three posterior to the clitellum. We may therefore conclude that the mid-body segments are triannulate as in *Abranchus*, *Ottoniobdella* and many other leeches (Mann, 1953). On that assumption there would be thirty-six annuli in the twelve segments between the clitellum and the posterior limits of the crop caeca. The remaining seven annuli are about the appropriate number for the segments of the anal region, since the number of annuli per segment is reduced towards each end of the body, in leeches generally. Allowing nine annuli for the clitellum, there are about eleven preclitellar annuli. There is, however, a tendency for annuli to be subdivided into two, particularly in the testicular region.

Internal anatomy

The following description is based on the series of transverse sections of a single specimen.

Body wall

The cuticle is thin (1–2μ) and smooth. The epidermis consists of a single row of cylindrical cells, 9–15μ × 5–8μ, with their inner edges flattened or less frequently slightly rounded and with their side-walls contiguous. The nuclei lie in the lower third of the cells. Ventrally the epidermal cells are generally somewhat smaller. The dermis is narrow, 12–25μ in thickness, and the fibrous connective tissue, so conspicuous in *Platybdella anarrhichae* (Leigh-Sharpe, 1916) is, as in *Abranchus* (Johansson, 1896 ; Knight-Jones, 1940), considerably reduced owing to dermal cells practically filling up the hypodermis. The dermal cells, which lack pigment granules, are flattened and measure 17–34×7–25μ. In places they are arranged two cells deep. Posteriorly, they become more spherical or rectangular in shape and tend to form a more or less continuous layer, as in *Abranchus microstomus* posteriorly and *Platybdella*

anarrhichae throughout the body. Their nuclei are usually flattened and measure 7–20×4–12μ.

The musculature generally consists of a single layer of circular muscle fibres, one or two layers of oblique muscle fibres and two to three layers of longitudinal muscle fibres. Sparse dorso-ventral fibres can occasionally be seen on each side of the gut.

Glands

(1) *Epidermal gland cells.* As in other Hirudinea, there are numerous gland cells lying in the epidermis and projecting into the hypodermis. These are not arranged in any definite manner. They are found on the anterior sucker and throughout the body, but are scanty on the clitellum and absent from the posterior sucker. Each gland cell is flask shaped, thin-walled and 12–15μ in diameter, with a narrow neck leading to the surface between the epidermal cells. The nuclei are oval, 7·5–12·5×3·7–7·5μ. They lie surrounded by a thin layer of cytoplasm in the basal portion of the cells, closely pressed against the cell walls. Their secretion is finely granular.

(2) *Sucker gland cells.* As in *Abranchus* (Johansson, 1896), *Branchellion*, *Platybdella anarrhichae*, *Platybdella michaelsoni*, *Crangonobdella murmanica* (Selensky, 1915), *Austrobdella translucens* (Badham, 1916) and others, numerous gland cells are present on the anterior sucker. These lie near the margin of the anterior sucker, mostly between the layers of circular muscle fibres, as in *Branchellion*. They are most numerous dorsally and dorso-laterally. Mid-ventrally, there appears to be present only a single pair of such gland cells. They are unicellular, thin-walled, flask shaped and large (22–37μ in diameter,) Their nuclei are spherical to oval, 7–12μ in diameter, and lie in the part of the cell remote from the duct. They are surrounded by a thin layer of cytoplasm and are generally pressed against the cell wall. Their ducts are long and narrow and open on the ventral side of the sucker. Their secretion is finely granular. No such gland cells are found on the posterior sucker.

(3) *Preclitellar gland cells.* These are 17–30μ in diameter and lie near the subdermal musculature. Most have a coarsely granular secretion distributed throughout the cytoplasm. The others have a definite secretion space filled with an homogenous secretion. The ducts open in two narrow lateral groups on the first and second preclitellar segments and both dorsally and laterally on the third preclitellar segment.

(4) *The clitellar gland cells* are smaller than in most Piscicolidae, being 7–32μ in diameter. They appear to be of three types :— (i) with a definite secretion space, filled with a granular secretion adjoining and indenting the nucleus ; (ii) as in (i) but with an homogenous secretion ; and (iii) with large oval nuclei and with the granular secretion distributed throughout the cytoplasm. These may be the same as type (i), but showing an earlier stage in the secretory cycle.

The first type are by far the most numerous. The second and third types are few and irregularly scattered amongst the first type. All three types extend from behind the clitellum to the base of the posterior sucker. Their

ducts are arranged in about nineteen bundles, each bundle carrying a variable number of ducts. The bundles lie irregularly, wherever space is available, just centrally to the clitellar gland cells. The ducts open to the exterior over the whole surface of the clitellum. Each appears to carry either homogenous or granular secretion throughout its length. Ducts carrying the two types of secretion occur together in the same bundle and open at the clitellum to the exterior independently of one another.

Gut

The proboscis is short, with its posterior end anterior to the first preclitellar segment. It has a very narrow lumen and the usual structure with radial, circular and longitudinal muscles. The circular muscle fibre layer is well developed, 5μ in thickness at the tip and nearly 12·5μ in thickness at the base. There are about seventeen bands of radial muscle fibres which spread out slightly fanwise on reaching the outer epithelium, but not to such an extent as to surround the longitudinal muscle fibres, as they do in *Austrobdella translucens*.

The salivary gland cells are large (75–120μ) and are found throughout the preclitellar region. Their nuclei measure 25–52μ and are indented by a secretion space, 17–40μ in diameter, which occupies the middle of the cell. The secretion is finely granular. Each transverse section shows three or four of these large cells, forming a group on each side of the oesophagus. The two groups do not meet either mid-ventrally or mid-dorsally. Their ducts enter the proboscis at its base and traverse its length to open at its tip, mostly running between its longitudinal and circular muscle fibres.

Immediately posterior to the proboscis, the gut lumen expands and remains wide until about the last preclitellar segment (segment 9—see p. 352). A pair of oesophageal glands is present in segments 10 to 11. These open into the oesophagus opposite the first clitellar ganglion through narrow ducts, each of which is provided with a small sphincter but no radial muscles. The glands are large pouches, much larger than the oesophagus itself at this level. They are lined by a single layer of flat epithelial cells, with large nuclei. Their walls are copiously folded and vascularised. The oesophagus is provided with a sphincter immediately anterior to the openings of the oesophageal ducts.

The crop is a simple tube (Plate 1 and Fig. 4A and B), without the paired lateral expansions so characteristic of most Piscicolidae. There is merely a slight widening of its lumen in each segment of the testicular region. Like the oesophagus, it is lined by a single layer of flat epithelial cells. Weak muscle fibres are occasionally to be seen on the outside of its wall.

The posterior crop caeca arise in the posterior part of segment 18. The crop is provided with a strong sphincter immediately anterior to their origin. They lie ventro-laterally to the adjoining intestine, and their epithelium is thin and slightly folded, consisting of a single layer of flat cells with large, oval nuclei. Anteriorly, in segment 19, these caeca fuse to form a common caecum which opens ventrally into the crop. Otherwise, the caeca remain separate throughout their length, but they approach one another closely at four points, once each in segments 19 and 20 and twice in segment 21. They extend to about segment 24.

There is another strong sphincter round the gut between the intestine and the crop. About eight pairs of lateral pouches have wide openings into the intestine. The first and second pairs are arranged more regularly than the remainder. The epithelium lining both the intestine and the pouches is similar to that of the crop, but more deeply folded.

The rectum is short with deeply folded walls. It becomes dorsal to open by the anus at the base of the posterior sucker.

The nervous system

The nervous system of *Sanguinothus pinnarum* possesses, instead of the usual twenty-one, only eighteen separate ganglia between the suboesophageal mass and the ganglionic mass of the posterior sucker. In an attempt to determine whether this reduction has occurred through fusion anteriorly or posteriorly, a careful study was made of the number of ganglionic capsules composing the suboesophageal mass.

It is generally accepted that a normal segmental ganglion in leeches contains six ganglionic capsules, one pair mid-ventrally, and a pair ventro-laterally on each side. In each pair, one capsule is placed behind the other, the six capsules in a normal segmental ganglion being, therefore, arranged in three longitudinal rows. Throughout the suboesophageal mass, however, the capsules are arranged in six longitudinal rows (Selensky, 1915). It is difficult to count their total number, but all writers are agreed that the suboesophageal mass innervates four segments. Mann (1953) recently studied the cerebral (supraoesophageal) "ganglion" in *Haemopsis sanguisuga* (Linnaeus) and found twelve ganglionic capsules. He concluded that the supraoesophageal mass of leeches is composed of two ganglia, and the head region, therefore, of six segments. The supra and suboesophageal masses are fused together, with no long circumoesophageal connectives.

In *Sanguinothus pinnarum*, the number of ganglionic capsules to be seen in single transverse sections through most of the length of the suboesophageal mass ranges from four to seven (Fig. 3A), but in the posterior part, for about a quarter of the total length of the suboesophageal mass, the number remains at three (Fig. 3B). It seems that a normal segmental ganglion, with its three rows of capsules, has become completely fused with the suboesophageal mass without a trace of a commissure to mark its anterior limit. This, therefore, would appear to correspond with the first preclitellar ganglion of other leeches, a conclusion which is confirmed by the positions of the clitellum and genital pores.

The last of the eighteen separate ganglia is very large. It is nearly 190μ in length, whereas the six ganglia preceding it measure 120, 110, 130, 90, 80 and 90μ in length respectively. The number of ganglionic capsules seen in one plane varies between three and four in this ganglion. It may therefore be regarded, provisionally, as a ganglionic mass resulting from fusion of the three anal ganglia of other leeches. Alternatively, it may have been formed by fusion of the two anterior ganglia of the anal region, the third having fused with the posterior ganglionic mass. The total number of capsules in the anal ganglion could not be counted accurately enough to decide between these possibilities, since the boundary membranes are not always distinct.

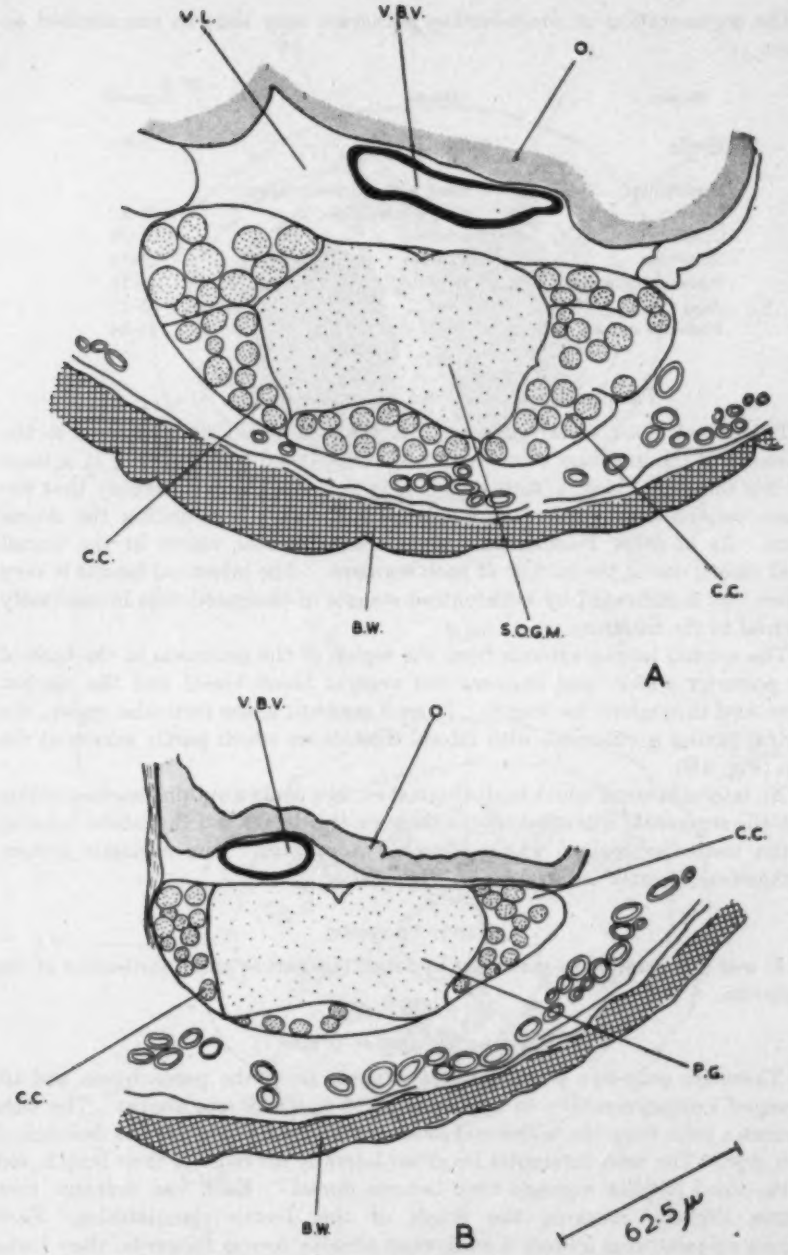

Fig. 3—*Sanguinothus pinnarum* sp. n. A, transverse section through suboesophageal ganglionic mass ; B, transverse section through first preclitellar ganglion.
BW, body wall ; CC, ganglion cell capsule ; O, oesophagus ; PG, first preclitellar ganglion ; SOGM, suboesophageal ganglionic mass ; VBV, ventral blood vessel ; VL, ventral lacuna.

The segmentation of *Sanguinothus pinnarum* may then be summarised as follows :—

Region	*Ganglia*	*Segments*
Head	6 fused	1–6
Preclitellar	1 fused with subœsophageal mass + 2 separate	7–9
Clitellar	3 separate	10–12
Testicular	6 separate	13–18
Posterior crop cæca	6 separate	19–24
Anal	3 fused	25–27
Posterior sucker	7 fused	28–34

The coelomic system and blood vessels (Fig. 4)

The dorsal blood vessel extends from the first preclitellar segment to the beginning of the intestine where it forms an intestinal blood sinus. It is large and fills the dorsal lacuna, so that it is only in a few places anteriorly that the lacuna can be distinguished as a continuous cavity surrounding the dorsal vessel. As in other Piscicolidae, there are segmental valves in the dorsal blood vessel, one in the middle of each segment. The intestinal lacuna is very narrow and is indicated by a discontinuous row of elongated cells immediately external to the intestine.

The ventral lacuna extends from the region of the proboscis to the base of the posterior sucker and encloses the ventral blood vessel and the ventral nerve cord throughout its length. In each segment of the testicular region, the ventral lacuna is enlarged, with lateral dilatations which partly surround the crop (Fig. 4B).

No lateral lacunae could be distinguished, nor could anything corresponding with the segmental communications between the dorsal and the lateral lacunae in the testicular region, which occur in *Abranchus*. The coelomic system is, therefore, greatly reduced, as in *Platybdella*.

Excretory system

It was not possible to make out in detail the nature and distribution of the nephridia.

Reproductive system (Plate 1)

There are only five pairs of testes. These lie in the parenchyma and are arranged intersegmentally in segments 14/15 to 18/19 (see above). The vasa efferentia arise from the testes mid-ventrally and open into the vas deferens of each side. The vasa deferentia lie dorso-laterally for most of their length, but in the third clitellar segment they become dorsal. Each vas deferens then dilates slightly, marking the origin of the ductus ejaculatorius. Each ductus ejaculatorius follows a somewhat sinuous course forwards, then turns ventro-posteriorly about the second preclitellar segment (segment 8). The terminal parts of the ducti ejaculatorii, which are still more dilated, begin in the

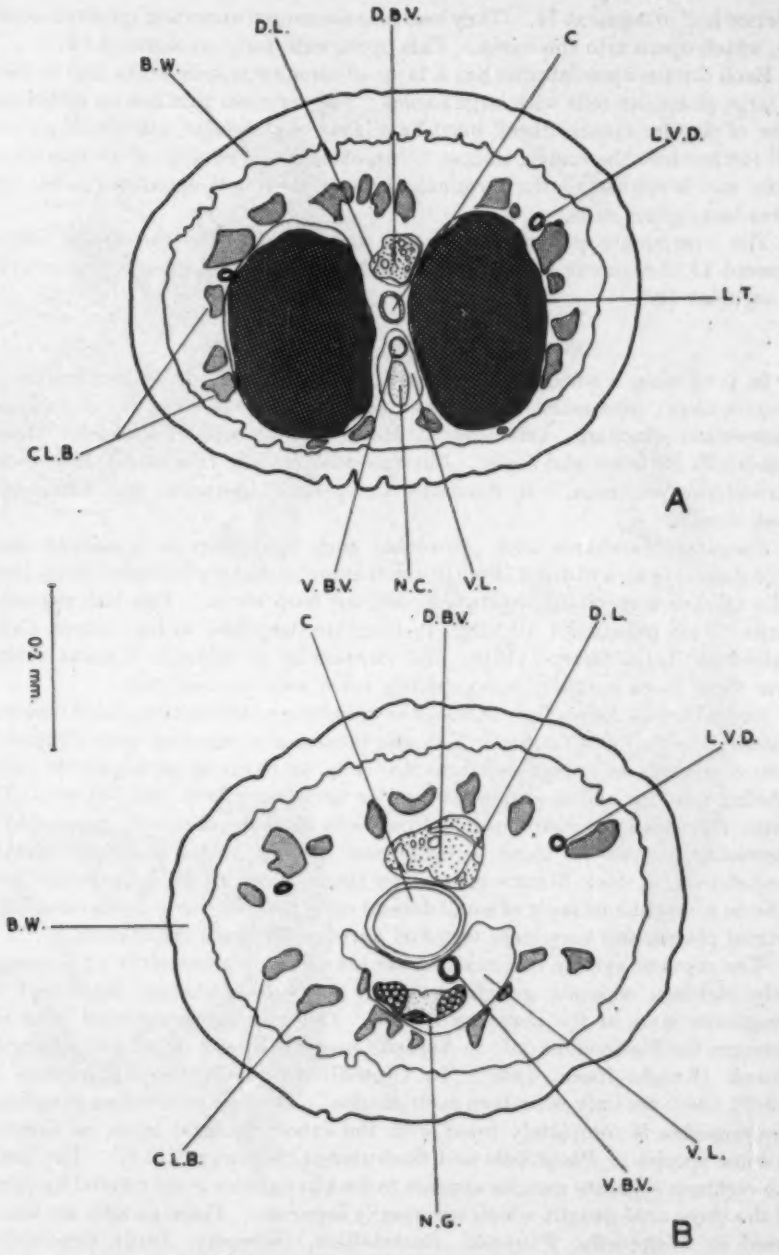

Fig. 4—*Sanguinobus pinnarum* sp. n. A, transverse section of body between 2nd and 3rd ganglia of testicular region ; B, transverse section through 4th ganglion of testicular region. BW, body wall ; C, crop ; CLB, clitellar bundle ; DBV, dorsal blood vessel ; DL, dorsal lacuna ; LT, left testis ; LVD, left vas deferens ; NC, nerve cord ; NG, nerve ganglion ; VBV, ventral blood vessel ; VL, ventral lacuna.

anterior half of segment 11. They lead into a common atrium or spermatophore sac, which opens into the bursa. This opens exteriorly on segment 11.

Each ductus ejaculatorius has a layer of circular muscle fibres and is lined by large glandular cells with large nuclei. The terminal part has an additional layer of circular muscle fibres, lined by a layer of glandular cells which project still further into the lumen, almost obliterating it. The wall of the spermatophore sac is similar to the terminal parts of the ducti ejaculatorii, but the bursa lacks gland cells.

The ovaries are paired, simple sacs and extend from the upper half of segment 11 to segment 14. The short oviducts unite and open to the exterior on segment 12.

COMPARISON WITH RELATED FORMS

In possessing a smooth skin devoid of any external metameric markings, *Sanguinothus pinnarum* resembles the genera *Abranchus* Johansson, *Austrobdella* Badham, *Ottoniobdella* Meyer and Moore, *Platybdella* Malm, *Hemibdella* Beneden and Hesse, *Marsipobdella* Moore, *Illinobdella* Meyer and *Piscicolaria* Whitman. It resembles the genera *Abranchus* and *Platybdella* most closely.

Sanguinothus shares with *Abranchus* such characters as a smooth skin, large dermal cells, a distinct clitellar constriction, a slightly flattened body, three pairs of eyes and wholly separated posterior crop caeca. This last character seems rather unusual. All other Piscicolidae described so far, except *Ganymedebdella* Leigh-Sharpe (1915) and *Arctobdella* de Silva & Kabata (1960), have these caeca partially or completely fused with one another.

Sanguinothus differs from *Abranchus* in having a still more reduced coelomic system, like that of *Platybdella*. It also possesses in common with *Platybdella* such characters as unpigmented dermal cells, an irregular arrangement of the clitellar bundles and a somewhat similar nervous system (see below). The genus *Platybdella* is distinguished from both *Sanguinothus* and *Abranchus* in possessing, despite its name, a cylindrical body, a rather indistinct clitellar constriction, a thick fibrous connective tissue layer in the hypodermis lying outside a continuous layer of small dermal cells, posterior crop caeca cohering in several places, and very large terminal parts to the ducti ejaculatorii.

The nervous system of *Sanguinothus pinnarum* is noteworthy in possessing only eighteen separate ganglia between the suboesophageal mass and the ganglionic mass of the posterior sucker. This number is recorded otherwise amongst the Piscicolidae only in *Nephelis lateralis* (Bristol, 1899) and *Abranchus blennii* (Knight-Jones, 1940). In *Cryobdellina bacilliformis* Brinkman Jr. (1948), there are only seventeen such ganglia. The first preclitellar ganglion of *Sanguinothus* is completely fused with the suboesophageal mass, as observed in some species of *Platybdella* and *Ozobranchus* (Selensky, 1915). The last of the eighteen separate ganglia appears to be a ganglionic mass formed by fusion of the three anal ganglia which are usually separate. These ganglia are almost fused in *Platybdella*, *Piscicola*, *Branchellion*, (Selensky, 1915), *Cryobdellina* (Brinkman Jr., 1948) etc. They are fused with the ganglionic mass of the posterior sucker in *Nephelis lateralis* (Bristol, 1899).

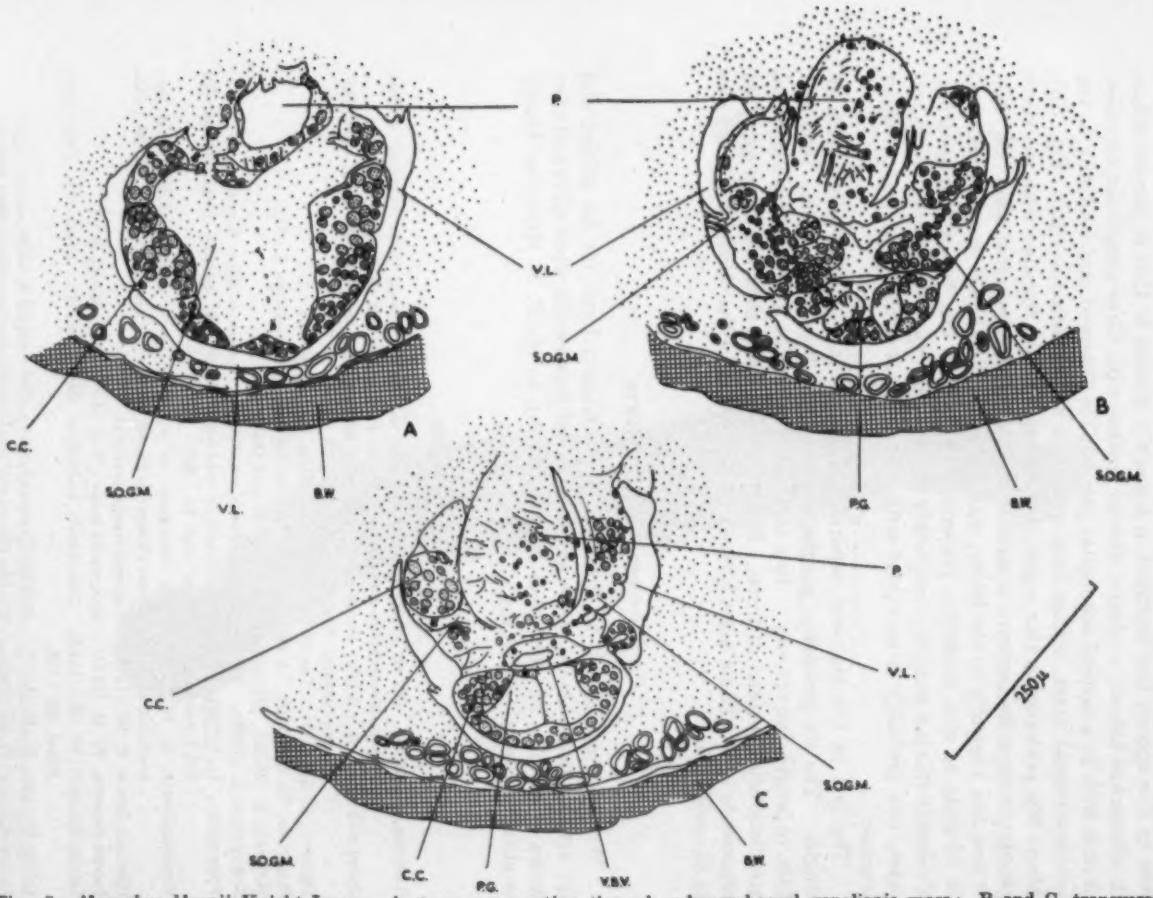

Fig. 5—*Abranchus blennii* Knight-Jones. A, transverse section through suboesophageal ganglionic mass; B and C, transverse sections through first preclitellar ganglion, B slightly anterior to C.
BW, body wall; CC, ganglion cell capsule; P, proboscis; PG, first preclitellar ganglion; SOGM, sub-oesophageal ganglionic mass; VBV, ventral blood vessel; VL, ventral lacuna.

A complete series of transverse sections of *Abranchus blennii* was studied by us in an attempt to determine whether the reduction in number of ganglia seen in this species had occurred in a manner similar to that in *Sanguinothus*. In *Abranchus blennii*, a central fibrous portion with three ganglionic capsules, as met with in a normal segmental ganglion, is observed mid-ventrally in the suboesophageal mass, about 140μ posterior to its anterior limit (Fig. 5B). About 30μ posterior to this region, the fibrous portion together with the three ganglionic capsules becomes separated from the suboesophageal mass (Fig. 5C). It now lies ventrally to the latter, with a thick-walled blood vessel in between. It extends in this condition posteriorly for about 60μ before disappearing. Evidently this is a ganglion completely fused anteriorly with the suboesophageal mass, but probably homologous with the first preclitellar ganglion of other leeches.

The last of the eighteen separate ganglia is large, being about 340μ in length. The six preceding ganglia measure 70μ, 210μ, 180μ, 200μ, 160μ and 140μ in length respectively. In a few sections, four to five ganglionic capsules are discernible instead of three. It is therefore likely that this large ganglion represents a ganglionic mass formed by the fusion of the three anal ganglia as in *Sanguinothus*.

ACKNOWLEDGMENTS

We are grateful (a), to Professor E. W. Knight-Jones for his advice and for making available (1) notes and sketches of this species when alive and (2) a series of sections of *Abranchus blennii* and (b), to Mr P. J. Miller for kindly sending us the specimens found at Port Erin.

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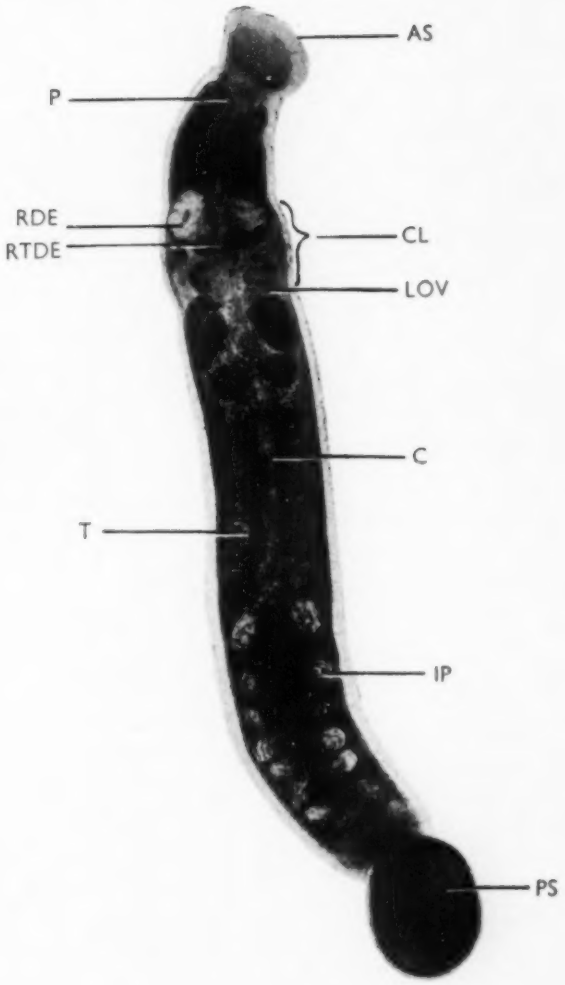

Sanguinothus pinnarum, sp.n. Photograph of a slightly flattened whole mount of gower-stained, balsam-mounted specimen, 12 mm. long. AS anterior sucker; C crop; CL clitellum; IP intestinal pouch; LOV left ovary; P proboscis; PS posterior sucker; RDE right ductus ejaculatorius; RTDE terminal part of right ductus ejaculatorius; T testis.

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THE FRONTAL SINUS OF THE GORILLA

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(With 1 plate and 6 figures in the text)

The development and topographical anatomy of the gorilla frontal sinus are described from late foetal life to senescence and details are given of its pneumo-osteolytic role in relation to that of the other paranasal sinuses. The adult sinus is shown to manifest one or other of two distinct morphological patterns and these to be determined by the more dominant maxillary sinus. Examples of frontal sinus perforative osteolysis are instanced and frontal sinus homologies are briefly discussed.

CONTENTS

	Page
Introduction	359
Materials and method	360
Ontogeny and topography of the frontal sinus	360
The frontal sinus during foetal life	360
The frontal sinus in infancy	360
The frontal sinus in the young	361
Immature and subadult stages	362
Adult stage	363
Pneumatic osteolysis of the frontal bone	366
Relative pneumatizing role of paranasal sinuses	369
Frontal sinus homologies	370
Acknowledgments	372
References	372
Material examined	373

INTRODUCTION

The individual paranasal sinuses of the gorilla, despite their morphological and functional importance, have been accorded surprisingly little systematic attention in the matter of their precise topographical disposition and their ontogenetic development. Admittedly the frontal and sphenoidal sinuses at least are almost invariably indicated in the sufficiently common illustrations of the sagittally divided gorilla cranium; but such delineations are necessarily inadequate as expressions of total sinus topography, whilst the specimens so depicted are usually taken from fully mature or from very old animals. To date no attempt appears to have been made to establish the successive ontogenetic growth stages of the frontal sinus, though Cave & Haines (1940) paid some attention to gorilla frontal sinus development in their study of anthropoid ape paranasal sinuses in general. In a series of seventeen gorilla crania ranging in age from infancy to senescence they noted the invariable suprabullar origin of the gorilla frontal sinus, the presence in young and in subadult material of never more than three (1 anterior, 2 posterior) ethmoidal sinuses (cells) and the constant numerical reduction or absence of ethmoidal cells

in adult specimens. The relevant literature then reviewed has been enriched in the interim by Wegner's (1956) isolated study of nasal fossa morphology in two adult gorilla skulls.

The present paper seeks therefore to amplify knowledge of the topographical anatomy of the gorilla frontal sinus, to demonstrate the successive stages of sinus development and to emphasise the important osteolytic role played by this sinus in the pneumatization of the gorilla cranium.

MATERIAL AND METHODS

Examination has been made, by dissection and/or observation, of the frontal sinus in a series of sixty-six gorilla skulls, ranging in age from late foetal life to extreme senescence. The series, particularized below, includes fifty adult crania (thirty-two males, eighteen females) preserved in the British Museum (Natural History) as well as certain no longer extant specimens, formerly in the Royal College of Surgeons Museum.

ONTOGENY AND TOPOGRAPHY OF THE FRONTAL SINUS

Expectedly, and as evidenced by the findings recorded below, the frontal sinus does not pursue an evenly progressive mode of expansion from the time of its initial appearance, but manifests instead periodic spurts of enlargement, which are to be correlated with the eruption of the temporary and permanent dentitions and the consequent rapid growth of the facial skeleton for the accommodation thereof and with the over-riding physiological necessity of obviating undue cranial-mass weight by a pneumatic lightening of cranial components. In addition, however, to such predictable physiological enlargement, the frontal sinus displays a propensity for steadily persistent expansion even after cranial growth is complete and the full permanent dentition has been acquired (i.e. throughout adult life into old age). These two aspects of frontal sinus expansion are considered below.

THE FRONTAL SINUS DURING FOETAL LIFE

Information is still wanting concerning the presence, order of appearance and disposition of the gorilla paranasal sinuses during the foetal period. Cave & Haines (1940) observed the invariable origin of the frontal sinus from a groove on the lateral nasal fossa wall immediately above the bulla ethmoidalis. In a male gorilla foetus (crown-rump length 240 mm.) approaching term Cave (1959 b) found this groove wholly devoid of the least expansion or diverticulum : a maxillary sinus was established and of the future fronto-ethmoidal complex a single posterior ethmoidal cell alone was present (Fig. 1 a).

THE FRONTAL SINUS IN INFANCY

Up to and including the time of eruption of the milk incisors a frontal sinus in the gorilla skull remains wanting. But *pari passu* with the acquisition of the full milk dentition the fronto-ethmoidal paranasal complex becomes fully and rapidly established to comprise a frontal sinus, an anterior ethmoidal sinus, and two posterior ethmoidal sinuses (cells). By the time the milk teeth have

fully erupted the frontal sinus has already extended to, or has even invaded, the base of the pars verticalis of the frontal bone (Fig. 1 b). Gross appearances in a series of young specimens suggest that the frontal sinus develops later than the two posterior ethmoidal sinuses and is itself followed by the appearance of the single anterior ethmoidal sinus.

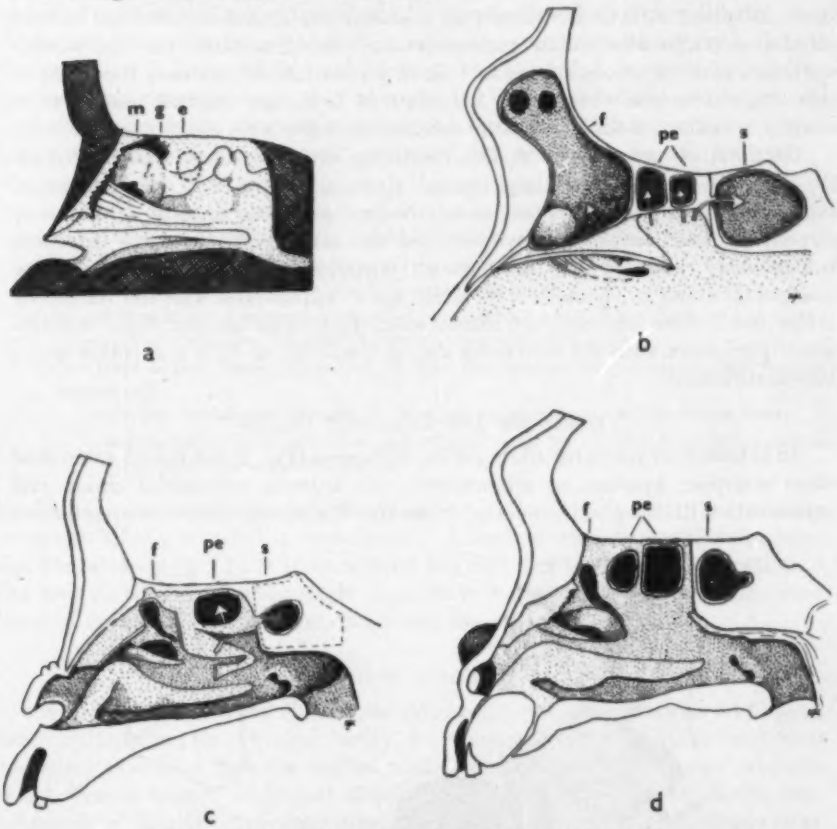

Fig. 1—The frontal sinus in the late foetal and infant gorilla.
In the foetus (*a*) the sinus is undeveloped : in (*b*) the milk dentition and all four permanent first molars have erupted : in the younger specimens (*c*) and (*d*) the milk dentition is present and the first permanent molars are in process of eruption.
e = ethmoidal sinus : f = frontal sinus : g = suprabullar groove: m = ethmoturbinal I : p.e. = posterior ethmoidal sinus : s = sphenoidal sinus.

The evidence of later foetal and of early postnatal specimens would therefore indicate that the neonatal gorilla skull is devoid of a frontal sinus, which makes its initial appearance during the period of eruption of the milk dentition.

THE FRONTAL SINUS IN THE YOUNG

By the time the first permanent molars begin to erupt the frontal sinus is well established within the frontal bone. As these teeth—the heralds of the

permanent dentition—continue to erupt the sinus undergoes a marked and rapid expansion. By about the fourth year of the animal's life, all four permanent first molar teeth have fully erupted and the sinus has extended vertically to the level of (but no higher than) the supraorbital torus, as well as posteriorly into the ethmoidal region proper. Already it manifests two or three incipient lateral diverticula of rounded conformation, and an antero-inferior downgrowth which surrounds and accommodates the intrusively upthrust anterior ethmoidal sinus. Save for its lateral recesses, the sinus at this stage does not extend far lateralwards from the sagittal plane—more strictly speaking, from the septum separating it from its contralateral fellow.

(It must be noted that, at this relatively early postnatal period, despite the presence of an expanding frontal sinus and of a full complement of ethmoidal sinuses, the maxillary sinus (the first paranasal sinus to appear) may already in some instances have invaded the ethmoidal region, to interpose contiguously between the hindermost posterior ethmoidal sinus and the sphenoidal sinus.) Thereafter, as adult age is approached and the remaining molar teeth come into use, the frontal sinus undergoes another rapid enlargement, *pari passu* with the increasing size of the skull, in both a superior and a lateral direction.

IMMATURE AND SUBADULT STAGES

In subadult or youngish adult gorilla specimens (Fig. 2) the fronto-ethmoidal sinus complex appears to advantage. An anterior ethmoidal sinus, still continuous with the middle meatus, excavates the lacrimal bone : two posterior

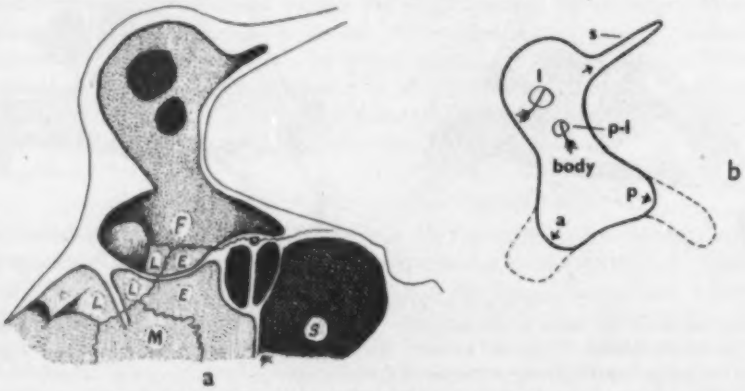

Fig. 2—Frontal sinus in the immature gorilla.

(*a*) Anterior ethmoidal sinus persists, but lacrimal and ethmoid bones already invaded by anterior diverticulum from frontal sinus. Two posterior ethmoidal sinuses present ; sphenoidal sinus considerably enlarged.

Superior, lateral and postero-lateral diverticula from the frontal sinus already well established and an incipient posterior diverticulum grows backwards above the posterior ethmoidal towards the sphenoidal sinus.

E=ethmoid : F=frontal : L=lacrimal : M=maxilla : S=sphenoid.

(*b*) Key to frontal sinus and diverticula : a=anterior, l=lateral, p=posterior, p-l=postero-lateral, s=superior.

ethmoidal sinuses (each extending to the medial wall of the orbit) remain contiguous to the sphenoidal sinus. The frontal sinus is now an extensive, irregular cavity, occupying not only the frontal bone but some part also of the lacrimal and of the ethmoid (i.e. its inferior portion has undergone appreciable expansion) : it presents also a number of smoothly rounded offshoots. Its mode of expansion—the outbudding of a succession of sacciform diverticula—is readily discernible from its interior architecture : for the mouth of each such diverticulum is bounded by two arching, smooth-boled, bony pillars, which by their symmetrical expansion towards the superficial table of the cranial roof confer upon the sinus interior a species of graceful fan-vaulting. Topographically, the cavity is broadly divisible into the following components :

(*a*) the body of the sinus, within the thick, inferior portion of the frontal bone, extending thence both upwards into the supraorbital torus and mediolaterally (from the interfrontal septum) behind the torus for some one-third the extent of the orbital roof ;

(*b*) a superior, or dorsal, extension, burrowing paramedially upwards and backwards into the floor, and towards the apex, of the trigonum frontale ;

(*c*) an infero-ventral, or anterior, extension, pneumatising parts of the lacrimal and of the ethmoid and containing the isolated anterior ethmoidal sinus ;

(*d*) two, three or more lateral extensions, of which two seem reasonably constant and proceed respectively

(i) directly lateralwards towards the external angular process of the frontal bone,

(ii) postero-lateralwards into that part of the frontal bone which floors the temporal fossa behind the crista temporalis.

This general arrangement of the sinus obtains in both sexes but is generally more obtrusively manifest in male crania. Already at this stage of development the frontal sinus may have so excavated the diploë of the retro-toral portion of the floor of the trigonum frontale as to leave but the thinnest possible outer layer of compact bone between itself and the exterior.

ADULT STAGE

As adult life is attained the frontal sinus commonly manifests an additional diverticulum from its principal cavity, i.e. a more or less horizontal (or infero-posterior) extension into the orbital roof, invading heavily the pars orbitalis of the frontal bone. The exact degree of ultimate development of this last extension is largely dependent upon the extent to which the naso-orbital region is pneumatized by the maxillary sinus and its topography is determined by the survival or disappearance of the posterior ethmoidal sinuses as the result of maxillary sinus activity. (For both the maxillary and the frontal sinuses appear to contend for the pneumatization of the naso-orbital region : in some gorilla specimens the maxillary sinus appears to be the dominant contestant, in others the frontal sinus. The maxillary sinus is commonly responsible for the absorption of the two posterior ethmoidal air sinuses, and, as noted, may precociously pneumatize the whole of the ethmoid bone. Occasionally, it would seem, a dominant frontal sinus may absorb one or other, but not both, of the two posterior ethmoidal sinuses. The topographical variations which result from the mutually antagonistic pneumatizing activities of the frontal and maxillary sinuses in the naso-orbital region are considered in detail below.)

The frontal sinus of the fully mature gorilla is a relatively enormous, multiloculate cavity, with a smoothly buttressed interior. Its capacity greatly exceeds that of the sphenoidal sinus and is but slightly inferior to that of the maxillary sinus (Fig. 3 a, b) and it maintains a steady, osteolytic expansion to the end of the animal's life. It occupies most of the vertical and horizontal portions of the frontal bone, the orbital roof and a variable part of the orbital

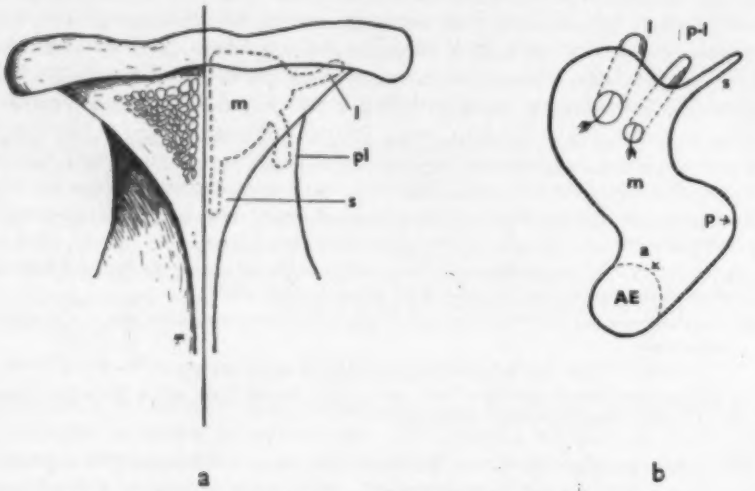

Fig. 3—Topography of adult gorilla frontal sinus (semi-diagrammatic).
The left half of (*a*) indicates the pneumatic 'sago' appearance of the trigonum frontale, the right half the topography of the main sinus cavity and its offshoots. In (*b*) the sinus is diagrammatised in vertical section.
AE = anterior ethmoidal sinus absorbed by a, the anterior diverticulum : m = main sinus cavity: l, p-l, p and s = lateral, postero-lateral, posterior and superior diverticula respectively.

wall. Topographically it is divisible into a main cavity and a number of constant, well marked diverticula, viz. superior (dorsal), lateral, postero-lateral, inferior (anterior) and posterior.

(*a*) The body of the sinus extends medio-laterally from the interfrontal septum to a point about half the distance therefrom to the lateral angle of the trigonum frontale. Antero-posteriorly it extends from the interior of the torus supraorbitalis backwards into the lower anterior moiety of the trigonum. It is separated from its contralateral fellow by an extremely thin interfrontal septum, which is but rarely deflected from the median sagittal plane.

(*b*) Its superior (dorsal) diverticulum extends paramedially from the main sinus cavity, upwards and backwards into the apical portion of the floor of the frontal trigone, replacing the diploic tissue thereof and interposing directly between the thinned outer and inner tables of the cranial vault.

(*c*) Its lateral diverticulum occupies the lateral angle of the trigonum frontale behind the lateral end of the supraorbital torus.

(*d*) The postero-lateral diverticulum (sometimes double) extends outwards and backwards from the main cavity, below the temporal ridge, to pneumatize that portion of the frontal bone which forms part of the floor of the temporal fossa.

(*e*) Its inferior (anterior) diverticulum, is a wide-mouthed cul-de-sac, often continuous, without constriction, with the main sinus cavity and extending antero-ventrally into the lacrimo-ethmoidal region. It extends lateralwards to the medial wall of the orbit, wherein, after preliminary osteolytic absorption of the single anterior ethmoidal sinus, it pneumatizes portions of the lacrimal and ethmoid bones, and inferiorly establishes contiguity to the bulla ossea nasolacrimalis.

(*f*) Its posterior diverticulum is inconstant. Its presence is conditioned by the extent to which a more functionally dominant maxillary sinus may establish itself as the principal pneumatizing agent in the posterior naso-orbital region. When present, its topography is further determined by the precise degree of persistence of the original two posterior ethmoidal sinuses. It is a wide-mouthed cul-de-sac, continuous uninterruptedly with the body of the frontal sinus, and carrying the parent cavity horizontally backwards towards the sphenoidal sinus. It never, however, invades the sphenoid bone itself, as may the maxillary sinus.

Both the frontal sinus and the more dominant maxillary sinus contend for the pneumatization of the medial orbital wall. In most gorilla crania the maxillary sinus exhibits a precocity of growth which enables it effectively to pneumatize this region at a relatively early stage, i.e. long before the frontal sinus attains its maximal development. Consequently a posteriorly directed pneumatizing diverticulum from the frontal sinus may be uncalled for and no such structure be developed. In other gorilla crania, however, the maxillary sinus is physiologically less dominant: it does not intervene between the frontal and sphenoidal sinuses by a pneumatization of the ethmoidal region, nor does it (usually) absorb the two posterior ethmoidal sinuses. Hence the final pneumatization of the posterior ethmoidal (nasal-orbital) region may be effected by one or other of two alternative methods, dependent upon the mutually antagonistic roles of the frontal and the maxillary sinuses, and macerated specimens reveal one or other of two patterns of local pneumatization.

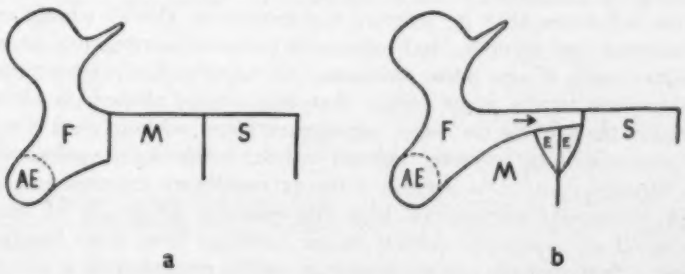

Fig. 4—The two varieties of adult gorilla frontal sinus (diagrammatic).

In (*a*) the sinus is limited by a dominant maxillary sinus, but in (*b*) is contiguous to the sphenoidal sinus by a posterior diverticulum.

AE = absorbed anterior ethmoidal sinus: E = posterior ethmoidal sinuses: F = frontal sinus: M = maxillary sinus: S = sphenoidal sinus.

The first pattern reveals a direct antero-posterior and successive contiguity of frontal, maxillary and sphenoidal sinuses: the frontal sinus is without posterior diverticulum, and has the bulla ossea nasolacrimalis immediately inferior, and the maxillary sinus immediately posterior, to it (Fig. 4 a).

The second pattern displays a posterior diverticulum of the frontal sinus establishing, above the maxillary sinus and the posterior ethmoidal cell(s), direct contiguity to the sphenoidal sinus (Fig. 4 b). The frontal sinus is contiguous inferiorly and successively to the bulla ossea nasolacrimalis, the maxillary sinus and the posterior ethmoidal sinuses, and posteriorly to the sphenoidal sinus.

The topographical disposition of the frontal sinus described herein for the fully mature gorilla skull obtains in either sex, though it is naturally seen to the greatest advantage in the tooth-worn, heavily crested crania of old male animals, wherein a relatively more massive facial skeleton demands, and receives, a relatively greater degree of compensatory pneumatization.

Apart altogether from exploration by partial dissection, by probing and by examination with transmitted light, the topography of the adult gorilla frontal sinus is commonly determinable with tolerable accuracy in skulls of either sex by direct naked eye inspection. For the thoroughly pneumatized parts of the trigonum frontale and of the roof and inner wall of the orbit manifest a curiously distinctive ' sago pudding ' appearance, due to the translucency and excessive thinness of the local outer compact bony layer. This ' sago ' appearance is best manifested by (though by no means confined to) the crania of large, elderly male animals. Excellent examples are specimens B.M. 36.7.14.1, B.M. 25.1.14.1, B.M. 25.1.4.3, B.M. 61.7.29.1, in each of which a paramedian ' sago ' area occurs bilaterally in the frontal trigone and a similar appearance characterizes the roof and most of the inner wall of each orbit. A comparable appearance may be encountered in the roof, floor and medial wall of the human orbit. Such a naked-eye surface ' sago ' appearance is characteristic of the intensely pneumatized temporal bone in the gorilla. All parts of this bone are so thoroughly pneumatized by a multiplicity of air cells from the several tympanic cell-series that its interior spongiosum is almost wholly replaced by air cavities and its endo- and exocranial layers of compactum are reduced to osseous sheets of rice paper thinness. So total, indeed, is temporal bone pneumatization in the adult gorilla that this cranial element is obtrusively delimited in the skull by its ' sago ' appearance alone, without need of reference to its circumscribing sutures, beyond which, incidentally, pneumatization never obtrudes. And it is because of the extraordinary thinness and fragility of their exocranial compactum that the mastoid temporals of macerated gorilla skulls so commonly exhibit minor breakage from mere handling and transport. Such damage as they sustain is readily recognizable as due to post-mortem injury and is never in the least suggestive of natural perforation of the outer compactum by the agency of the mastoid air cells within.

PNEUMATIC OSTEOLYSIS OF THE FRONTAL BONE

In full maturity or in old age a natural pneumo-osteolytic perforation of the compact outer table of the frontal bone may result from the persistent absorptive activity of the frontal sinus, and single or multiple deficiencies may make their appearance in the floor of the trigonum frontale (see Fig. 5). Such non-pathological perforation was observed, in varying degrees of severity, in three out of fifty-four adult gorilla crania specifically examined, all from male

animals. In none of these three crania is there the least suggestion of bone-loss from ante-mortem trauma or disease (e.g. sinusitis, osteomyelitis, neoplasm) nor any evidence of local post-mortem damage during or since their maceration. Simply, at the sites of perforation, the excessively thinned exocranial compactum flooring the trigonum frontale has undergone absorption through the osteolytic activity of the frontal sinus epithelium, permitting that epithelium to establish direct contact with the exocranial periosteum.

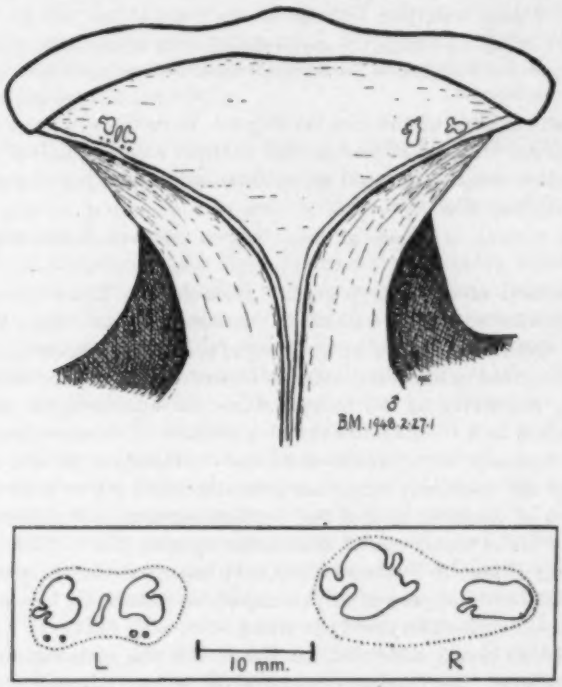

Fig. 5—Bilateral frontal sinus osteolysis in an adult gorilla (B.M.1948.2.27.1). Dorsal aspect of cranium showing multiple perforations in the trigonum frontale and (inset) details thereof to scale.

The first of the three specimens manifests perforative pneumo-osteolysis bilaterally, the second and third unilaterally (sinistrally) as follows :

Specimen 1. B.M.1948.2.27.1. Large ad. male. Sagittal crest 34 mm., lamboid crest 36 mm., at inion. Maxillary incisors mere stumps : old-standing fracture of right canine with occlusal surface now flush with that of the cheek teeth : p.m. 1, 2 and m. 1, 2 with enamel-denuded occlusal surfaces.

On the left, the outer area of the frontal trigone, immediately contiguous to the superior temporal line, presents a thinned, translucent area (11 mm. transversely and 6 mm. anteroposteriorly) rendered cribriform by three large and four small openings. The transverse and anteroposterior dimensions of the major

deficiencies are respectively—of the lateral deficiency 4 mm. × 4·5 mm., of the intermediate deficiency 2·5 mm. × 1 mm., and of the medial deficiency 4·5 mm. × 3·5 mm. The edges of the gaps are sharp : their outlines are shown in Fig. 5. The four minor deficiencies lie behind the major ones and are mere pin-hole non-vascular perforations in the bone. The corresponding right region of the trigonal floor presents a semi-translucent area (6 mm. long by 10 mm. wide) perforated by an irregularly oval gap of some 4 mm. by 6 mm. About 8 mm. medial thereto appears a second deficiency, of irregular outline, 7 mm. long × 8 mm. wide (see Fig. 5).

Specimen 2. B.M.1948.5.4.2. Large ad. male. Sagittal crest 13 mm., lambdoid crest 19 mm., at inion. Teeth less worn than in preceding specimen, but the lingual moieties of m. 1 and 2 crowns enamel-denuded.

In the left lateral area of the frontal trigone, immediately contiguous to the superior temporal line, a non-mensurable thinned area presents a single sharp-edged deficiency, irregularly oval in outline and measuring some 4 mm. long by 6 mm. wide (see Fig. 6).

Specimen 3. B.M.1948.4.1.1. Large ad. male. Sagittal crest 36 mm., lambdoid crest 36 mm., at inion.

In the left lateral area of the trigonum frontale is a minute, rounded, non-vascular perforation (ca. 1·5 mm. in diameter) with thin, sharp edges, immediately anterior to the superior temporal line (not figured).

These specimens afford dramatic evidence as to the persistent lifelong pneumatizing proclivity of the frontal sinus, to the osteolytic potency of its lining epithelium and to the subservient plasticity of osseous tissue.

A physiologically comparable pneumatic osteolysis of the maxilla and premaxilla by the maxillary sinus has been described (Cave 1959 a) in the two extant species of elephant and of the vertical portion of the palatine bone by the maxillary sinus in all recent rhinoceros species (Cave 1957). In various cetacean forms Fraser & Purves (1958) have reported the successive stages of the manifestation of an essentially comparable osteolytic absorption of the pterygoid bone by the pterygoid air sinus.

The condition herein described for the gorilla was seemingly noted earlier, but misinterpreted, by Duckworth (1904) in an examination of 109 gorilla crania from British and French museums : for, without stating their numerical incidence, Duckworth referred in his series to certain examples of what he regarded as ulcerative changes ' on the external angular process of the frontal bone '. There is no known or sufficient pathological cause for ' ulceration ' in this region and the very topography of Duckworth's imagined ' lesions ' points to their being, in fact, frank examples of frontal sinus perforative osteolysis.

The conveniently descriptive term ' pneumatic osteolysis ' (Cave 1959 a) may require explanation. Pneumatic osteolysis is defined as that process whereby an invading air-containing cavity (paranasal sinus, tympanic cell or cell group) effects a progressive pneumatization of a cranial element (or elements) involving, in greater or lesser degree, absorption both of its interior diploic tissue (spongiosum) and of its exterior compact bone (compactum). The essential nature of this highly complicated process remains obscure, as does the controlling mechanism of its initiation and maintenance.

The effective pneumatizing agent responsible for the profound internal architechtonic transformation of the cranial bones concerned would appear to be the epithelium of the so-called 'muco-periosteum' lining the invading sinus or cell, the air content of the resultant cavity being apparently a mere sequela of epithelial activity.

The successive stages of the establishment of a particular paranasal sinus may be traced (as herein for *Gorilla*) in a suitable age-range of material, and accelerations of sinus growth can be correlated with growth spurts in the development of the facial skeleton occasioned principally by the successive eruption of the deciduous and permanent dentitions. Such morphological studies, however, throw no light upon the essential—and doubtless hormonal—machinery of pneumatic osteolysis.

RELATIVE PNEUMATIZING ROLE OF PARANASAL SINUSES

It may here be convenient to summarize the mutual pneumatizing roles of the several gorilla paranasal sinuses, both minor (ethmoidal) and major (maxillary sphenoidal, frontal).

The ethmoidal sinuses make their appearance following establishment of the maxillary sinus. They are small and never more than three (one anterior, two posterior) in number. The anterior ethmoidal sinus may invade the lacrimal bone, but the posterior ethmoidal sinuses never obtrude beyond the limits of the ethmoid bone. The anterior ethmoidal sinus ultimately disappears by absorption by the frontal sinus. The two posterior ethmoidal sinuses may persist, but generally the foremost member of the pair, and not infrequently both sinuses, undergo absorption by the maxillary sinus.

The sphenoidal sinus, although a major member of the series, is significantly less 'dominant' in the pneumatization of the skull than either the frontal or the maxillary sinus. Its pneumatizing activity is confined to the basis cranii (i.e. to the body, great wings and pterygoid processes of the sphenoid) outside which limits it never obtrudes. Thus it attempts no encroachment upon the adjacent and vulnerable ethmoidal region, nor any absorption of the posterior ethmoidal sinuses lying in direct anterior contiguity. Contrariwise it not uncommonly suffers diminution from an intrasphenoidal encroachment by the more 'dominant' maxillary sinus, which, having first pneumatized the entire naso-orbital ethmoidal region, extends backwards to invade the corpus sphenoidale itself.

The maxillary sinus, ontogenetically the first, and topographically the most extensive, of the series, is unmistakably the most 'dominant' of the gorilla sinuses. In young adult life it already pneumatizes, in greater or lesser degree, the maxilla, palatine, maxillo-turbinal and ethmoidal bones, and with increasing age it encroaches further into the entire naso-orbital region, absorbing the foremost, sometimes both, of the two posterior ethmoidal sinuses and even invading the sphenoid bone itself. It contends with the frontal sinus for the pneumatization of the orbital medial wall and by precocious occupation thereof may render impracticable the development of a posterior diverticulum from the frontal sinus, thus determining in large measure the ultimate topography of that sinus.

The frontal sinus, at first confined to the frontal bone, pneumatizes that element most efficiently, even to the extent of localized perforative absorption of its exocranial compactum (*ut supra*). It rapidly extends into the lacrimo-ethmoidal region and with advancing age invariably absorbs the single anterior ethmoidal sinus. By a variable posterior diverticulum into the pars horizontalis of the frontal bone it may establish contiguity with the sphenoidal sinus, never, however, invading the sphenoid bone. Such contiguity is possible in those instances only wherein the maxillary sinus is less ' dominant ' than usual and pneumatizes relatively less of the naso-orbital ethmoidal region.

The initially narrow naso-orbital region of the gorilla skull is thus primarily pneumatized by the ethmoidal sinuses, which undergo reduction or even total obliteration from a secondary pneumatization of the region effected by the frontal and maxillary sinuses in mutual antagonism. This characteristic arrangement in *Gorilla* is the direct contrary of conditions in *Homo*, wherein an initially wide naso-orbital region is pneumatized by an abundance of ethmoidal cells, which continue to proliferate and to extend topographically throughout life.

FRONTAL SINUS HOMOLOGIES

In the homologization of paranasal sinuses it is axiomatic that the topographical relations of the ostium of any particular sinus constitute the sole criterion of sinus definition. Every paranasal sinus is morphologically and developmentally a diverticulum from the nasal fossa, hence its true identity is established by the site of its exit therefrom (i.e. by the mutual relationships of the structures circumscribing its ostium) and not at all by the subsequent topography of the fully developed sinus itself, which may well involve, by extension, cranial elements other than the one whereafter it is conveniently named.

Unfortunately, in this connexion convenience of reference rather than morphological exactitude has greatly influenced nomenclature, so that paranasal structures of widely divergent morphological pattern are included under the same name. Thus the supra-orbital paranasal vacuity in *Lemur*, the so-called ' frontal ' sinus, is demonstrably not homologous with the topographically correspondent supra-orbital cavity, a true frontal sinus, in *Pan* and *Gorilla*.

A ' true ' frontal sinus, in the hominid sense, can obtain only when ethmoidal sinuses are developed, since such a structure is fundamentally nothing more than the most anterior member of the ethmoidal sinus complex devoted essentially to pneumatization of the frontal bone. It was shown (Cave & Haines, 1940) that ethmoidal sinuses in Primates are restricted to *Pan*, *Gorilla* and *Homo*, and that consequently these forms alone possess a true frontal sinus : also that, so far as is known, the ontogenetic origin of the gorilla frontal sinus derives invariably from the supra-bullar groove of the middle meatus of the nasal fossa. Such a supra-bullar origin may occur also in *Homo*, but only as the rarest of several alternative modes of origin.

A descriptively ' frontal ' sinus is necessary to, and is therefore present in, many Primate and non-Primate forms. But in *Homo*, *Pan* and *Gorilla* the

frontal region is pneumatized, essentially, by the most anterior member of an ethmoidal sinus complex, derived (in whatever manner) from the middle meatus of the nasal fossa. In *Homo* the frontal sinus remains topographically intra-frontal, while the ethmoidal cells proper proliferate continuously and extend their territory throughout life. In *Gorilla*, on the contrary, the frontal sinus rapidly and invariably enlarges extra-frontally, while the associated three ethmoidal cells suffer potential or actual reduction. The impression is gained therefore that the initial complement of gorilla ethmoidal sinuses is insufficient for its allotted mechanical function of pneumatizing the medial orbital wall.

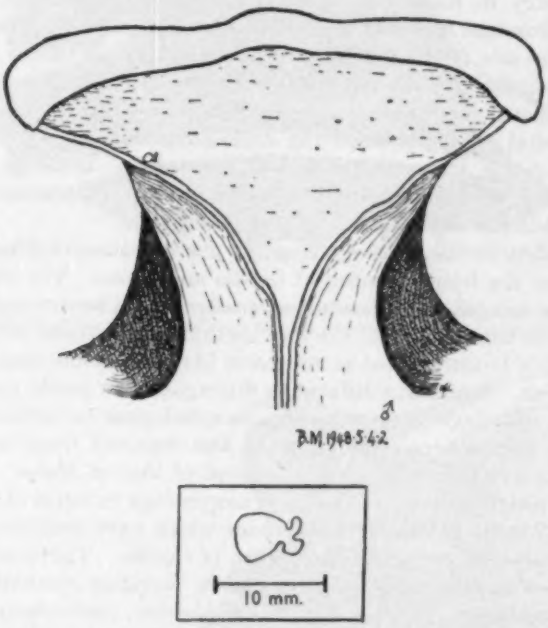

Fig. 6—Unilateral frontal sinus osteolysis in adult gorilla (B.M. 1948.5.4.2). A single perforation occurs in the left portion of the trigonum frontale and is shown to scale in the inset.

The numerical disparity of ethmoidal cells between *Homo* and *Gorilla* is so striking as to require special consideration, for this apparently minor morphological difference may not be without value as an indication of past phylogenetic trends in the two forms. Accepting the general assumption that the Primate stock evolved from more generalized mammalian progenitors, it has been shown (Cave, 1948) that the profound structural revolution occasioned by the conversion of the nasal fossa of a generalized mammal into that of a primitive Primate involved, *inter alia*, loss of the hinder portion of the ancestral nasal chamber (with corresponding loss of ectoturbinals and reduction of endoturbinals), and reduction, or even suppression, of the old inter-orbital

region and its replacement by an inter-orbital septum. Such an early Primate type of nasal fossa is retained by *Tarsius*, *Cebus*, *Saimiri*, *Alouatta*, *Macaca*, *Papio*, *Cercopithecus* and *Colobus*. It seems undeniable that cerebral enlargement and a concomitant expansion of the supportive cranial base has occurred independently in the three Primate groups of the Platyrrhini, the Catarrhini and the Anthropoidea. In both the Platyrrhine and the Catarrhine groups the more primitive and smaller-brained extant members thereof still retain an inter-orbital septum, but in the larger-brained members of each group this septum is lost. In the Anthropoidea, the degree of cerebral enlargement attained by *Pan* and *Gorilla* is exceeded by that attained by *Homo* alone. Phylogenetically in these two closely-allied African apes, the eyeballs and orbits have remained constant in size but the latter have become more widely separated : the site of the lost primitive inter-orbital septum is now occupied by the secondarily invasive nasal fossa, which has undergone some degree of secondary expansion towards that end. But neither in *Pan* nor in *Gorilla* has the inter-orbital region attained the dimensions characteristic of *Homo* : in both of these ape forms therefore less naso-orbital tissue is available for pneumatization and consequently a lesser number of pneumatizing agents (ethmoidal cells) is developed.

It is possible, on the basis of a common manifestation of ethmoidal sinuses, to homologize the frontal sinuses of *Gorilla* and *Homo*. Yet the embryology of the gorilla frontal sinus remains virtually still unknown and the striking contrast in the constitution of the fronto-ethmoidal complex in the two forms might plausibly be interpreted as indicative of nothing more than an evolutionary parallelism. Significant differences distinguish the gorilla nasal fossa from the human, and there is no evidence, morphological or palaeontological, to suggest that *Gorilla* ever attained (much less departed from) an inter-orbital region of the width and proportions typical of that of *Homo*. It is possible therefore to interpret human nasal fossa morphology in terms of a phylogenetic avoidance of those particular vicissitudes which have befallen, and are now indelibly impressed upon, the nasal fossa of *Gorilla*. The available evidence being thus so largely equivocal, the greater therefore remains the need for further detailed study of the gorilla nasal chamber, particularly in embryonal and foetal material, and for the determination, *inter alia*, of the precise ontogenetic development of the gorilla frontal sinus.

ACKNOWLEDGMENTS

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Fig. 1.—Mucosal adult female gorilla cranium, in sagittal section. Showing type A frontal sinus (F) separated from sphenoidal sinus (S) by a dominant maxillary sinus (M).

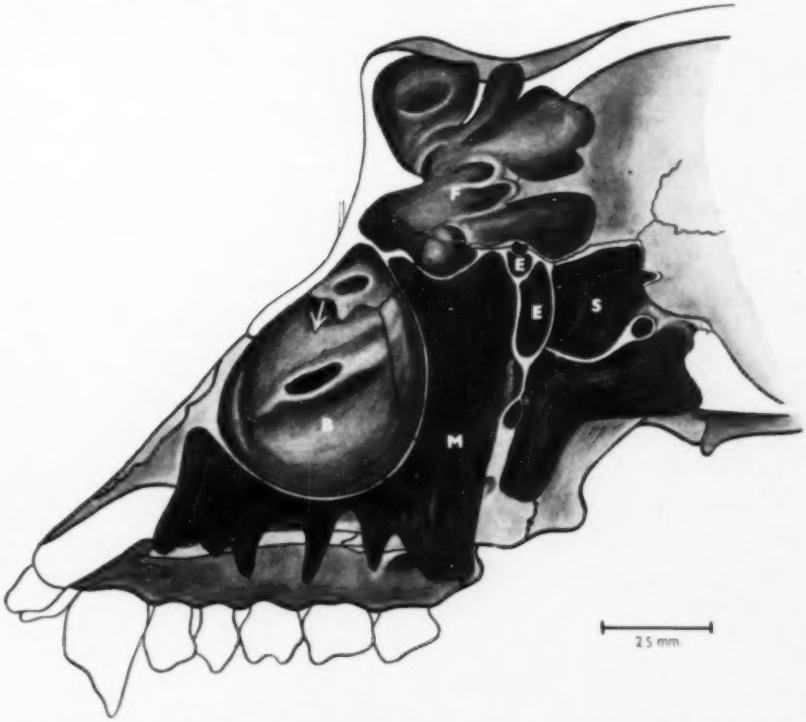

Fig. 2.—Macerated adult male gorilla cranium, parasagittally dissected. Showing relationship of type B frontal sinus (F) to nasolacrimal bulla (B), maxillary sinus (M), persistent posterior ethmoidal sinuses (E, E) and sphenoidal sinus (S).

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MATERIAL EXAMINED

Mucosal specimens (6)

Foetus, ♂, near term	Private coll., R.223	Young (4 yrs), milk dentition plus 1st perm. molars	St. Thomas's Hosp. Anat. Dept. (deposited)
Infant, milk dentition	St. Thomas's Hosp. Anat. Dept. (deposited)	Immature	Roy. Coll. Surgs. Mus., Phys. Ser. E 108.5
Young, milk dentition plus 1st perm. molars	Private coll., R.58	Adult, ♀, 11 yrs.	Roy. Coll. Surgs. Mus., uncatalogued

Macerated specimens (60)

Infant, milk incisors only	Roy. Coll. Surgs. Mus., Comp. Ost. 34.4	Adult ♂	B.M. 23.11.29.1
Young, milk dentition	Roy. Coll. Surgs. Mus., Comp. Ost. 30A	Adult ♂	B.M. 35.3.19.1
Young, milk dentition	Roy. Coll. Surgs. Mus., Comp. Ost. 30.31	Adult ♂	B.M. 25.1.4.3
Young, ♀, 1st perm. molars	Private coll. C 1.2	Adult ♂	B.M. 29.12.29.1
Young ♂	B.M.29.1.1.2	Adult ♂	B.M. 23.11.29.2
Immature ♀	Roy. Coll. Surgs. Mus., Comp. Ost. 27	Adult ♂	B.M. 22.2.10.1
Immature ♀	B.M. 10.11.27.1	Adult ♂	B.M. 29.1.1.1
Adult ♂	Roy. Coll. Surgs. Mus., Comp. Ost. 25	Adult ♂	B.M. 49.663
Adult ♂	Roy. Coll. Surgs. Mus., Comp. Ost. 20	Adult ♂	B.M. 1951.9.27.19
Adult ♂	Roy. Coll. Surgs. Mus., Comp. Ost. 22.2	Adult ♂	B.M. 36.7.7.1
Adult ♂	St. Thomas's Hosp. Anat. Museum	Adult ♂	B.M. 55.28.4.1919
Adult ♂	Private coll., C 1.1	Adult ♂	B.M. 1864.12.1.13
Adult ♂	B.M. 25.1.4.2	Adult ♂	B.M. 1948.3.3.2
Adult ♂	B.M. 13.2.2.2	Adult ♂	B.M. 1948.4.1.1
Adult ♂	B.M. 7.1.8.1	Adult ♂	B.M. 1948.5.4.2
Adult ♂	B.M. 35.12.16.1	Adult ♂	B.M. 1948.2.27.1
Adult ♂	B.M. 48.436	Adult ♂	B.M. 1948.5.4.1
Adult ♂	B.M. 78.12.14.1	Adult ♀	B.M. 1948.3.31.1
Adult ♂	B.M. 23.11.29.6	Adult ♀	B.M. 1948.4.1.2
Adult ♂	B.M. 13.2.2.1	Adult ♀	B.M. 1948.12.20.2
Adult ♂	B.M. 20.4.13.4	Adult ♀	B.M. 1948.3.31.2
Adult ♂	B.M. 61.7.29.1	Adult ♀	B.M. 1951.9.27.20
Adult ♂	B.M. 48.435	Adult ♀	B.M. 1951.9.27.17
Adult ♂	B.M. 1011E	Adult ♀	B.M. 1951.9.27.18
Adult ♂	B.M. 36.7.14.1	Adult ♀	B.M. 1951.9.27.16
Adult ♂	B.M. 25.1.4.1	Adult ♀	B.M. 1951.9.27.12
		Adult ♀	B.M. 64.12.1.1
		Adult ♀	B.M. 23.11.29.5
		Adult ♀	B.M. 7.1.8.6
		Adult ♀	B.M. 23.11.29.8
		Adult ♀	B.M. 7.1.8.3
		Adult ♀	B.M. 7.1.8.4
		Adult ♀	B.M. 7.1.8.7
		Adult ♀	B.M. unregistered

THE ANATOMY OF THE ANOMODONT *DAPTOCEPHALUS LEONICEPS* (OWEN)

BY

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[Accepted 11th October 1960]

(With 5 plates and 10 figures in the text)

A general description of the skull of a particularly well-preserved specimen of *Daptocephalus leoniceps* (Owen) is given and the surface features of the septomaxilla described; it is suggested that this bone may have supported a nostril-closing valve and that *D. leoniceps* may have been slightly amphibious. The conformation of the bones in the pterygoid region is described and attention is drawn to an arrangement which may prove to be a useful systematic character. A description of the masticatory apparatus is given and its mode of action is discussed, as are the homologies and significance of the labial fossa. The auditory region is described. A description of a small bone, interpreted as an ossified extra-stapes, is given and the position of the tympanum is discussed. The proatlas, atlas and axis are described and a restoration of the neck muscles is made. Some of the bones of the manus are described and the functional significance of a number of the characteristics of the genus *Daptocephalus* is briefly outlined.

CONTENTS

	Page
Introduction	375
General anatomy of the skull	376
The septomaxilla	382
The side walls of the braincase and the pterygoid complex	382
The palate and masticatory apparatus	385
The auditory region	390
The occiput, cervical vertebrae and neck muscles	392
The manus	398
Functional considerations	399
Acknowledgments	401
References	401
Key to abbreviations of names of bones used in the figures	402
Explanation of the plates	402

INTRODUCTION

The specimen of *Daptocephalus leoniceps* (Owen) about to be described, number 349 in the Bernard Price Institute collection, consists of an extremely well preserved skull and lower jaw with the first three cervical vertebrae and a number of the bones of the right manus. In the skull some bone has been eroded away from the front of the snout and mandible, while on the left side the tusk is broken off and the posterior portion of the zygomatic arch and much of the occipital plate are missing. The whole right side of the skull, more than half the occiput with the condyle, the dorsal surface and the palate are, however, intact and well preserved. The specimen came from near Escourt in Natal, in an area where upper Cisticephalus zone is overlaid by

Lystrosaurus zone : it consisted of a single block of matrix no longer *in situ*, but washed out in a donga. Although its original site could not be found, there is no reason to suppose that it did not come from the Cisticephalus zone, the usual horizon for *Daptocephalus leoniceps*.

The preservation of the specimen is so good that it has been possible to make out a number of anatomical details which add to our knowledge of this species and are relevant to the understanding of anomodont anatomy in general. These points will be dealt with in detail in what follows. A general description of the skull will first be given, for although *D. leoniceps* is a reasonably common species, good figures of the skull are not easily available. Owen's (1876) catalogue is a rarity and Broom (1932) figures only the preparietal region and Toerien (1953) the palate.

GENERAL ANATOMY OF THE SKULL

Daptocephalus leoniceps is one of the largest of the anomodonts, specimen B.P.I 349 having a basilar length of 423 mm. (see Table 1 for measurements). The skull clearly belonged to an old animal and by no means all of the sutures can be made out with certainty. It shows the characters typical of advanced members of the group : the postorbital is much longer than the preorbital region ; the inter-temporal width is much less than the inter-orbital, the temporal crest being formed by the vertical edges of the parietals overlaid by the postorbitals which slope slightly outwards and downwards on either side (see Figs. 1 and 2 and Pl. 1). Behind the parietal crest the wings of the squamosal extend backwards, diverging only slightly, so that, viewed from above, the skull has the same long narrow appearance as the *Kannemeyeria* figured by Pearson (1924 a). In the present specimen there has been some lateral compression of the posterior part of the skull and the squamosal wings almost certainly diverged more widely so that the true shape would have been more triangular, as in the *Kannemeyeria erithea* Haughton figured by Case (1934).

The zygomatic arch is strong with extensive overlapping between the squamosal, which extends almost as far as the middle of the orbit, and the jugal, which reaches back almost to the level of the posterior end of the parietal crest. Beneath the orbit the maxilla is prolonged backwards on the outside of the bar to overlap both the squamosal and the jugal. As suggested by Pearson (1924 a) this extensive overlapping probably has the function of strengthening the zygomatic arch so as to counteract the weakening of the middle part of the skull caused by the extreme length of the temporal fossae and the narrow parietal crest.

There is some unclarity about the condition of the postfrontal in *D. leoniceps*. The bone which Owen (1876) figures as the postfrontal is clearly part of the postorbital. Haughton (1932), describing a specimen from Tanganyika which he considers to belong to a distinct but related species, says that there is no postfrontal. In this character, he says, the skull agrees with one in the South African Museum which is " a typical *Daptocephalus leoniceps*, according to Dr. R. Broom, and differs from the type of the species which is in the British Museum ". In the present specimen the postfrontals

Table 1—Dimensions of specimen B.P.I. 349. All measurements in millimeters.

Skull	
Maximum length, from front of snout to posterior edge of squamosal	520
Length from anterior edge of premaxilla to back of occipital condyle	423
Length from anterior edge of premaxilla to back of internal quadrate condyle	435
Length from anterior edge of premaxilla to anterior edge of pineal foramen, measured along the curve	320
Length from anterior edge of pineal foramen to the point of divergence of the parietals	130
Maximum width across postorbitals	246
Interorbital width	99
Width across lower border of postorbitals at posterior end of pineal foramen	45
Height from lower edge of pterygoid to top of parietal crest at level of pineal foramen	195
Maximum height, from tip of squamosal to edge of external quadrate condyle	304
Length from anterior edge of premaxilla to front of internal palatine tubercles	155
Length from posterior edge of inter-pterygoid fossa to back of occipital condyle	150
Minimum width across pterygoids on palate	48
Mandible	
Maximum length, from anterior edge to back of glenoid	375
Maximum depth, at posterior end of symphysis	135
Length from anterior end to posterior end of symphysis measured along the curve on the outer surface	230
Atlas	
Maximum length of body of neural arch	55
Height of body of neural arch	42·5
Maximum length of intercentrum	ca. 50
Axis	
Length of body of centrum (excluding odontoid)	48
Width of body of centrum	40
Maximum height	110
Maximum width of odontoid articulating surface	72·5
3rd vertebra	
Length of centrum on ventral surface	41
Width of posterior end of centrum	60
Height of posterior end of centrum	57·5
Stapes	
Length along middle of ventral surface	34
Width across middle of ventral surface	23·8
Diagonal distance from centre of extra-stapedial facet to tip of proximal articulation	48
Extra-stapes	
Length	16
Width of shaft	10
Height of shaft	6·5

appear perfectly clearly on the skull roof as two narrow strips of bone extending forward, one on either side of the preparietal, from a point just in front of the pineal foramen to within about 12 mm. of the walls of the orbits (see Fig. 2). On the inside of the posterior wall of the orbit the postfrontal appears again

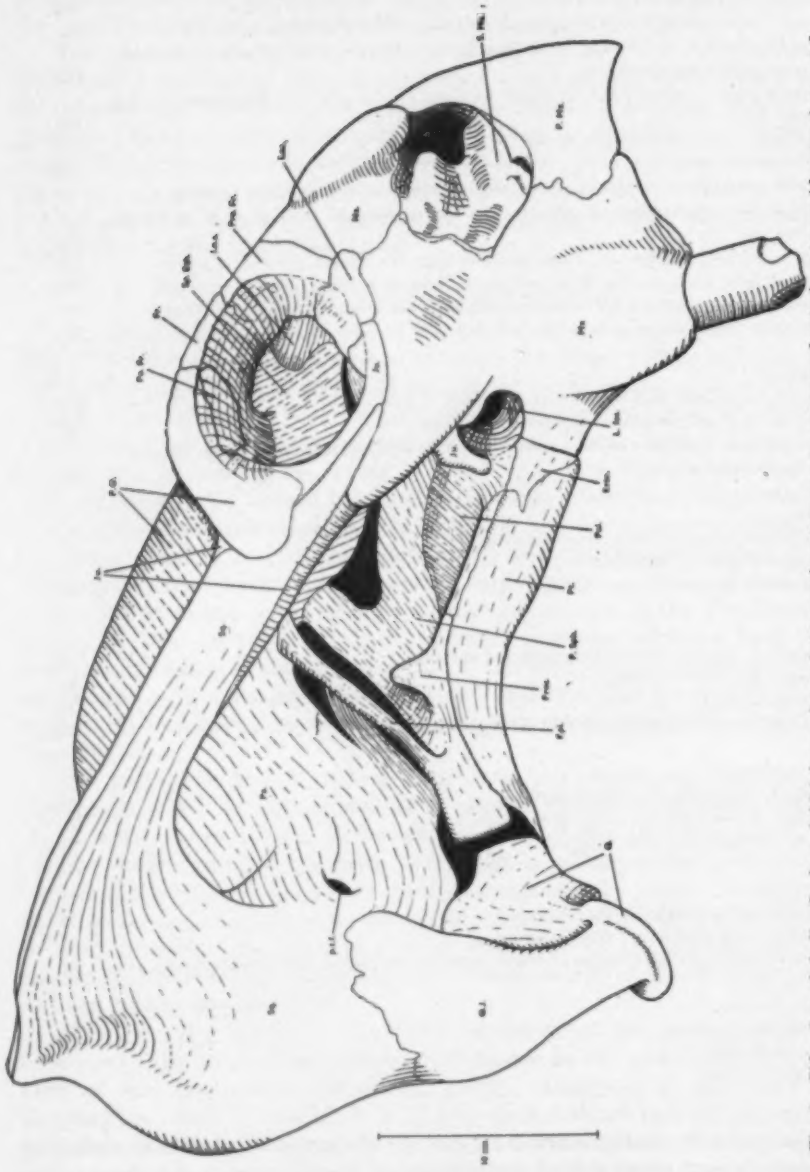

Fig. 1—Side view of skull. L. foss., labial fossa; i.n.s., inter-nasal septum; proc., process of anterior pterygoid extension; p.t.f., post-temporal fossa. (For abbreviations of names of bones see page 402.)

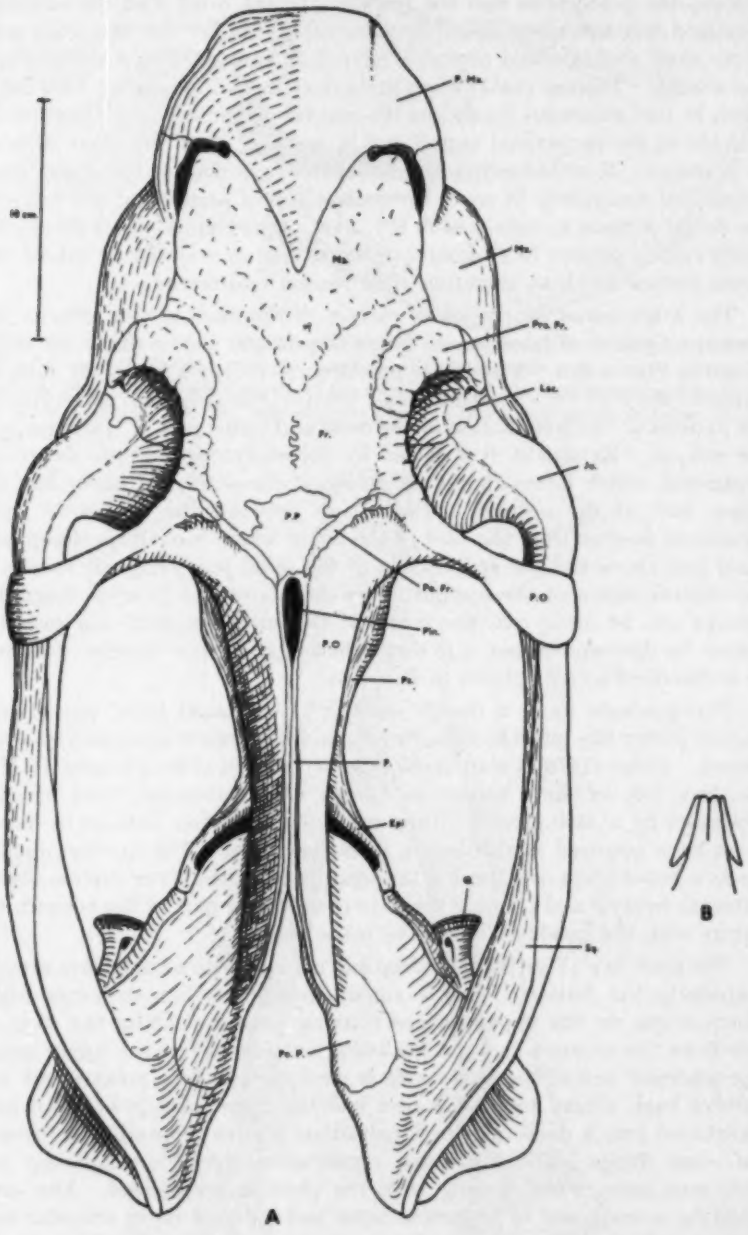

Fig. 2—A, dorsal view of skull ; B, section through middle of parietal crest. Pin., pineal foramen.
(For abbreviations of names of bones see page 402.)

between the postorbital and the frontal. In the orbit wall the sutures are ill-defined and difficult to make out and were it not for the very clear sutures on the skull roof it would probably have been concluded that the postfrontal was absent. Toerien (1953) has shown that in two species of *Dicynodon* of which he had numerous specimens the sutures on the skull roof are extremely variable in the prepariетal region and it is likely that the same is true of *D. leoniceps*. It would require but a slight overgrowth of the postorbitals or prepariетal completely to cover the narrow slip of postfrontal left exposed on the dorsal surface in specimen B.P.I. 349. It therefore seems likely that a postfrontal is present in *D. leoniceps*, but it may or may not be visible on the dorsal surface and may therefore often remain undetected.

The other bones on the outer surface of the skull in the anterior region present no points of interest. As far as the sutures can be made out they are shown in Figs. 1 & 2. Posteriorly on the occiput only a few of the sutures are distinct (see Fig. 8). A rather small postparietal joins the posterior end of the parietal at the termination of the crest and forms part of the upper wall of the occiput. Externally it is joined by the enormous occipital flange of the squamosal, which forms almost the whole of the outer and about half of the upper wall of the occiput. The suture between the opisthotic and the squamosal is clear from the base of the latter where it overlaps the quadrato-jugal just above the jaw articulation to the small post temporal vacuity. In the central region of the occiput, however, above the foramen magnum, no sutures can be made out, the limits of the supra-occipital and exoccipitals cannot be determined, nor is it clear whether a separate tabular was present, as is described by Cox (1959) in *Kingoria*.

The quadrate forms a double condyle of the usual form, consisting of a narrow pulley-like internal and a broad external condyle separated by a narrow groove. Owen (1876) describes the external condyle of his specimen (B.M.N.H. No. S.A. 56) as being almost as narrow as the internal, from which it is separated by a wide groove. It seems likely that some damage or distortion must have occurred in this region in his specimen. The quadratojugal runs up as a broad plate overlapping the squamosal on the outer surface above the external condyle and forming the edge of the lower part of the occiput, but its suture with the quadrate cannot be made out.

The lower jaw (Figs. 3 & 4) is deep and massive with an extensive symphysis. Anteriorly the dentary forms a square-ended beak-like structure with two sharp ridges on the upper surface running backward along the edge of the jaw from the anterior end and flattening out just before the upper margin of the posterior end of the symphysis is reached. From a point about 20 mm. further back almost to its posterior end the upper margin of the dentary is excavated into a deep, narrow longitudinal groove separating an inner from an outer flange. The functional significance of this arrangement will be discussed later, when dealing with the chewing mechanism. The articular condyle is composed of a narrow inner and a broad outer articular surface, corresponding to the inner and outer quadrate condyles on the skull. Below the articulation is a short but stout retro-articular process.

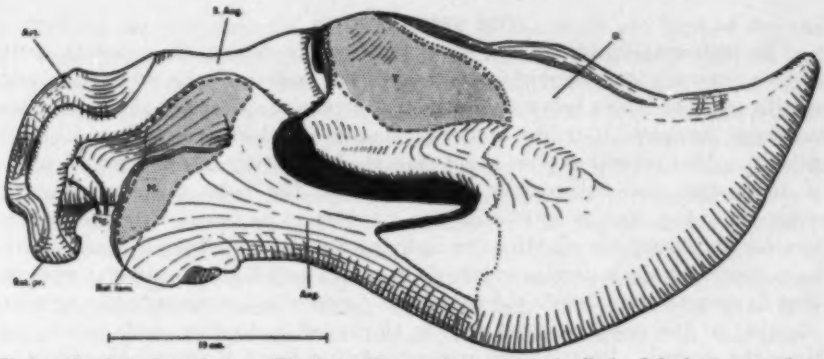

Fig. 3—Mandible, lateral view. The dotted line marks the posterior limit of the horny covering ; the stippled areas, T. and M., show the insertions of the temporalis and masseter muscles and the arrows, Ptg., indicate the position of the attachment of the pterygoideus anterior. Ref. Lam., reflected lamina ; Ret. pr., retro-articular process. (For abbreviations of names of bones see page 402.)

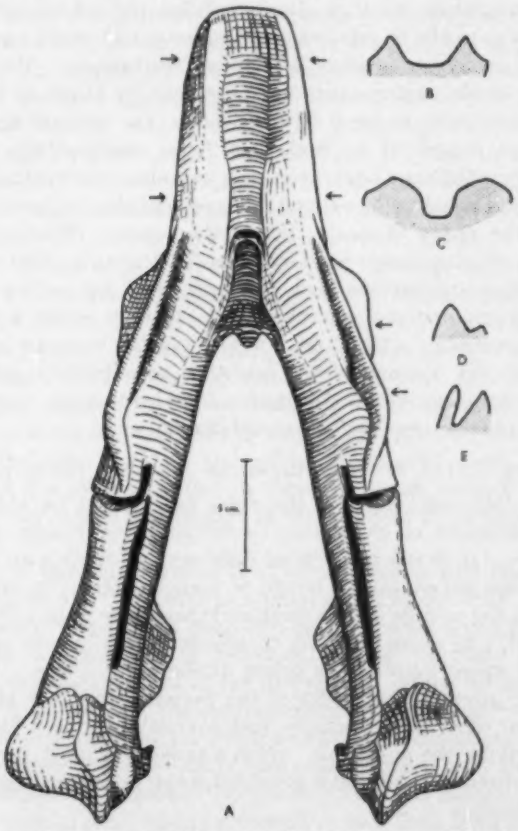

Fig. 4—A, mandible seen from above. B–E, sections through the edge of the jaw at the levels shown by the arrows.

THE SEPTOMAXILLA

The septomaxilla lies in the usual position, bordering the external naris postero-ventrally and sloping inwards from the surface of the premaxilla and maxilla, so that it lies below the level of the general surface of the snout. Its structure, however, is unlike any septomaxilla of which I have found a description. Its external surface is not smooth, but thrown into a complex series of depressions and tuberosities (see Plate 1). The large size and complex structure of the bone at once raise the question of its functional significance. Devices for closing the nostril occur in living reptiles. Bellairs & Shute (1953) have described the muscular valve of Crocodilia and Stebbins (1948) mentions that in desert-living lizards the cavernous tissue which surrounds the anterior chamber of the nasal passage may be thickened to form a valve which can close the nostril. Pratt (1948) notes that this form of nostril closure may occur in aquatic as well as in deserticolous lizards. It seems possible that in *D. leoniceps* the septomaxilla may have formed the anchorage for some type of nostril closing valve. In the Crocodilia the muscular closing valves are not associated with any peculiarities in the structure of the septomaxilla ; it is therefore most likely that in *Daptocephalus* the postulated closing device was operated as in the lizards, by erectile tissue and would have been similarly covered by a protective layer of keratinized epithelium. Although the surface complexities of the septomaxilla were not seen by Owen in his specimen and he did not recognize it as a separate bone, the general appearance of the external naris suggested to him that " the tegumentary covering of the infundibular nostril may have been of a valvular construction ".

If, indeed, a nasal valve existed in *Daptocephalus* its function was probably to prevent the entry of water, rather than sand. This is not intended to suggest that *Daptocephalus leoniceps* was fully aquatic, but it may well have been somewhat amphibious and at times have fed on aquatic vegetation. This would have involved putting the snout under water, a habit which may well have occurred in a fairly close relative of the more aquatic *Lystrosaurus*. The fact that the opening of the nostril is considerably higher on the skull than in the less specialized *Dicynodon* also lends some support to the idea that *Daptocephalus* was slightly amphibious.

THE SIDE WALLS OF THE BRAINCASE AND THE PTERYGOID COMPLEX

Dorsally, the side walls of the skull are formed by the almost vertical parietals overlapped on either side by the posterior process of the postorbital. The lower border of the postorbital does not end flush with the parietals but overhangs, like the eaves of a house, to form a distinct inverted groove which is highest in the middle of the crest and dies away anteriorly and posteriorly (see Fig. 2B). It seems difficult to interpret this groove otherwise than as forming the upper limit of the origin of the jaw muscles. This implies that not only the upward facing edge of the parietal crest, but also its downward-sloping sides, were free of muscle, and covered only by a layer of connective tissue underlying the epidermis. Such a projecting crest, devoid of any easily damaged structures, may have provided some protection for the more deeply situated muscles.

Behind the epipterygoid the side walls of the skull are formed by the parietal, postparietal and supraoccipital extending downwards towards the bones of the otic region : anterior to the epipterygoid the bones of the basicranial axis extend upwards and there is also ossification in the ethmoid region. The homologies of the various bony elements in this latter area are not fully agreed upon and a complex terminology exists. Agnew (1958) has studied this point in *Dicynodon grimbeeki* Broom and discusses the matter at some length. In the present specimen the sutures in this region cannot be seen, so that it throws no light on the question of homologies. It will therefore be simplest to adopt the terminology used by Cox (1959) in describing *Kingoria:* He interprets those elements rising upwards from the basicranial axis as a single parasphenoid-basisphenoid complex and those arising in the interorbital region as an undivided sphenethmoid complex.

In *Dicynodon grimbeeki* the bony side walls of the skull are very incomplete : large gaps exist in the anterior wall of the orbit in front of the sphenethmoid complex, between it and the epipterygoid and between the latter and the supraoccipital and otic region. In *Kingoria* the walling in is more extensive and the gaps are smaller : the orbit is walled in anteriorly, the gap between parietal and prootic is small, but the unossified space in front of the epipterygoid is still quite large. In *Daptocephalus* the process has gone further and the walling in is more complete than in any other anomodont in which this region is fully known (see Fig. 1). The specimens of *Kannemeyeria* investigated by Pearson (1924 a) and by Case (1934) did not allow the structure of the side walls of the braincase to be studied but one would expect that in this genus, in some respects even more specialized than *Daptocephalus*, the walling in would be no less extensive. In *Daptocephalus* the downward flange of the parietal meets the prootic, leaving only a narrow slit behind the epipterygoid and above the pila antotica. The space in front of this pillar-like epipterygoid is similarly reduced to a narrow slit by upward extension of the parasphenoid-basisphenoid to meet the sphenethmoid. Ventrally the parasphenoid-basisphenoid expands to meet the palatine, obliterating the gap between these two bones which exists in *Kingoria*. Anteriorly the cultriform process of the parasphenoid again meets the ethmoid so that there is a space, presumably for the exit of the optic nerve, between the two parasphenoid-ethmoid zones of contact. The dorsal wall of the orbit is walled in mesially by an ossified interorbital septum, lying slightly mesial to the part of the ethmoid plate which contacts the parasphenoid.

The condition of the epipterygoid is of some interest and presents some problems of interpretation. Starting as a narrow rod at the level of the postorbital bar it extends downwards from the parietal with a slight backward slope and expands ventrally into a footplate butting ventrally against the dorsal edge of the quadrate process of the pterygoid. So far the arrangement described is quite orthodox, but the conformation of the region lying anterior to the footplate is unusual and its interpretation problematical. Figure 5 illustrates the condition of the whole of this region, which may conveniently be termed the pterygoid complex. The pterygoid is an elongated plate of bone forming the ventral limit of the side wall of the skull from its posterior

contact with the quadrate to its anterior end where it just contacts the maxilla at the ventral corner of the small ectopterygoid. At the posterior end a discontinuity separates the pterygoid from the footplate of the epipterygoid (Fig. 5 A). This discontinuity corresponds with the expected position of the

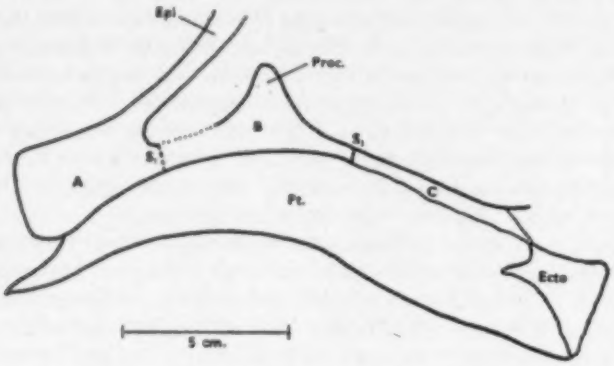

Fig. 5—Pterygoid complex of right side ; anterior end to the right. For further explanation see text.

suture between pterygoid and epipterygoid but instead of turning dorsally to delimit the footplate anteriorly in some such position as S1, it continues along the length of the pterygoid to end anteriorly at the posterior corner of ectopterygoid, thus dividing off a dorsal slip of bone, A, B, C, from the pterygoid. This discontinuity is present in exactly the same position on both sides of the specimen, a fact which, while not excluding the possibility of its being a post mortem crack rather than a suture, makes this interpretation less probable. A second discontinuity, S2, which may or may not be a suture, divides off the thin triangular anterior slip, C, from the middle portion, B, and the footplate, A. The middle portion, B, is prolonged dorsally into a blunt, rounded process, proc., and between this and the ascending limb of the epipterygoid the bone, sloping inward, is continuous with the parasphenoid-basisphenoid complex. No discontinuity can be detected either at S1, between the footplate, A, and the middle section, B, nor between B and the parasphenoid-basisphenoid. While A clearly belongs to the epipterygoid, the regions B and C cannot be identified with any certainty. There are three problems: (i) are B and C separate bones, or is the discontinuity S2 merely a crack? (ii) Is B to be regarded as an anterior extension of the epipterygoid, or is it part of the parasphenoid-basisphenoid? (iii) If C is indeed a separate bone, what is its origin? It seems simplest to assume that S2 is an artifact, leaving only a single bone, B+C, to be accounted for. Case (1934) has described a similar but much less extensive bone in *Kannemeyeria erithea* and regards it as an anterior extension of the epipterygoid. This appears to be the most likely interpretation as it is easy to visualize a gradual forward extension of the epipterygoid footplate in the course of evolution. The functional significance of the extension may be the same as that of the forward

extension of the squamosal in the zygoma, to wit, to compensate for the weakening of the middle region of the skull accompanying the narrowing of the parietal region. If this is correct, then we should expect to find the anterior pterygoid extension developed only in genera in which there is a narrow parietal crest : moreover, the degree of forward extension shown may provide a systematic character of value at the specific level since rapid evolutionary change must have been occurring in this region. In any case, it is clear that the pterygoid complex deserves detailed investigation in advanced anomodonts both to establish the homologies of its various components and also to investigate its possible systematic value.

THE PALATE AND MASTICATORY APPARATUS

The structure of the palate has been described by Toerien (1953). In the present specimen the sutures are not visible in the region of the posterior end of the premaxilla and anterior end of the palatines but elsewhere they agree with Toerien's description (see Fig. 6).

Anteriorly the edges of the premaxilla and maxilla form sharp cutting edges across the front of the snout and along its sides as far as the tusks. On its palatal surface the anterior part of the premaxilla curves downwards and bears two well-marked anterior palatal ridges separated by a deep groove. In Toerien's figure (1953, Fig. 58) the palatal ridges appear as two rounded bars dividing the front of the mouth into three grooves of equal depth. In specimen B.P.I 349 the condition is different : here the ridges are the sharp sides of a central groove and on either side of them the surface of the palate runs almost horizontally for a little way and then curves down to the side of the mouth as shown in Fig. 6 B. The maxilla joins the premaxilla about half way between the anterior end of the snout and the tusk. It expands somewhat on the palatal surface mesial to the tusk and extends behind it to make a short contact with the pterygoid, lateral to the palatine. Posteriorly, on its more horizontal portion, the premaxilla forms a thick rounded median palatal ridge which runs back to the anterior margin of the internal nares, forming the posterior process, and is continuous with the anterior end of the vomer. The vomer is a slim vertical sheet of bone running back between, and at a higher level than, the pterygoids ; it divides the interpterygoid space into right and left halves. Posteriorly it forks and on either side joins the antero-medial process of the pterygoid.

On either side of the deep interpterygoid fossa the pterygoids form vertical flanges, extending considerably further ventrally than the vomer. Behind the fossa the pterygoids meet and are overlaid ventrally by the basisphenoid, while anteriorly they diverge slightly and the posterior wings of the palatines form flat plates applied to their mesial surfaces. Figure 6 C shows a section across the palate at the point of bifurcation of the vomer. At the antero-external margin of the pterygoid lies the small triangular ectopterygoid. Anteriorly the palatines spread out ventrally to form horizontal plates which are in contact with the maxilla antero-laterally and form the side walls of the internal nares. According to Toerien (1953) there is a short contact between premaxilla and palatine at the anterior margin of the choanae.

The structure of the horizontal surface of the palatine and the adjacent part of the maxilla is of some interest. On this surface, between the tusks and the choanae, are a series of bony knobs resembling small teeth. These knobs are arranged in two groups, an inner and an outer, on either side and they will be referred to as the internal and external palatal tubercles. There has been some distortion in this region and the arrangement of the tubercles is not absolutely symmetrical on the two sides, but there is no doubt that they

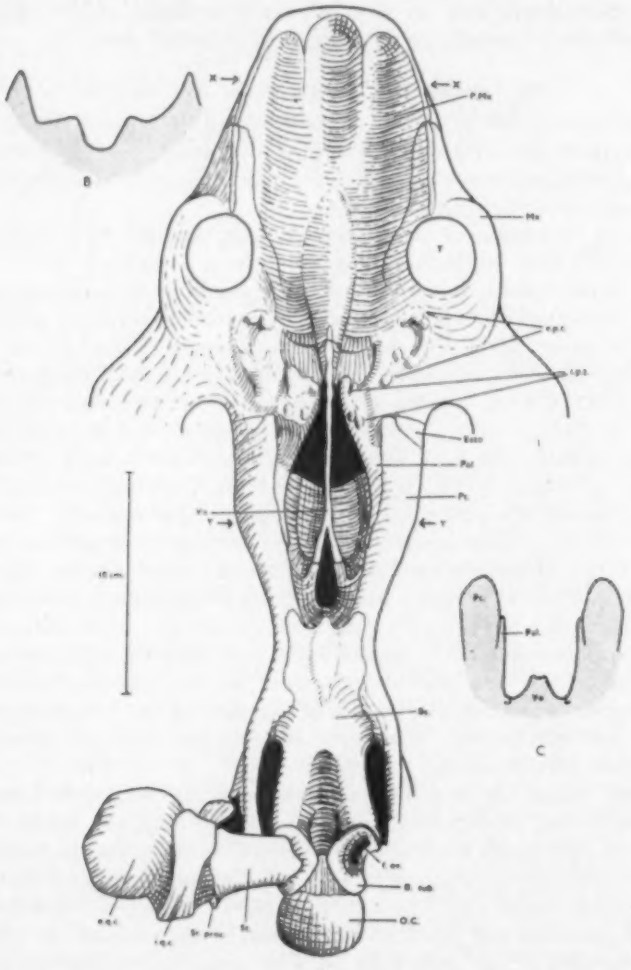

Fig. 6—A, palatal view of skull ; B and C, sections at the levels of the arrows XX and YY respectively B. tub., basi-occipital tuber ; e.p.t., external palatal tubercles ; e.q.c., external quadrate condyle ; f. ov., fenestra ovalis ; i.p.t., internal palatal tubercles ; i.q.c., internal quadrate condyle ; St. proc., process on stapes for articulation with extra-stapes ; T., tusk.

represent a genuine structure, not a pathological condition. The left hand tubercles seem to be the better preserved. Anomodonts are generally considered to have possessed a horny beak. Judging from the roughness of the bone, the horny covering must have extended over the palatal tubercles and there can be little doubt that they formed part of a chewing mechanism. Preservation is frequently poor in this region and it seems likely that palatal tubercles may have been present in other species of anomodont, although they have not been recorded. In *Kingoria* Cox (1959) describes a pair of sharp ridges in very much the same position as the palatal tubercles : these may have formed a food chopping or crushing device functionally equivalent to the tubercles.

Any discussion of the function of the palatal tubercles requires consideration of the occlusal relations of the jaws. Figure 7 shows sectional views through upper and lower jaws in three positions, at the anterior end of the mouth near the back of the anterior palatal ridges and in two positions through the palatal tubercles. There has been some lateral compression of the specimen and this has probably crushed in the rami of the lower jaw rather further than the more solid skull.

Anteriorly the narrow lower jaw fits inside the wider premaxilla. The sides of the snout are concave downwards and the edges of the mandible concave upwards, so that the jaws must have met first along the anterior margin. As they did so, the sharp edges of the upper jaw and the anterior ridges on the crests of the dentary would have approached each other, acting as a pair of cutters. In Fig. 7 A the transverse distance between upper jaw edge and dentary ridge seems rather excessive, even when allowance is made for the horny covering, but it may have been less in life, since it is not possible to judge the degree of lateral compression undergone by the mandible. The function of the anterior palatal ridges remains obscure : even if they bore sharp ridges of horn, the upward curvature of the dentary would seem to prevent their approaching the edges of the lower jaw sufficiently closely for them to have formed an auxiliary cutting device.

The canine bears wear facets at its tip antero- and postero-externally. These cannot have been made by wear against the lower jaw, and they may indicate some use of the tusks in food collection, as has been suggested by Pearson (1924 a) in *Kannemeyeria*.

The two groups of palatal tubercles lie above the region of the mandible where the upper surface of the dentary is formed by two flanges separated by a deep groove. These seem to fit reasonably well against the surfaces covered by the palatal tubercles. The crushing which has occurred makes the orientation in the lateral plane of the upper and lower jaw sections relative to each other a matter of guess work. The relations shown in Fig. 7 B & C where the better preserved left-hand tubercles have been used, are judged to be the most probable. If we imagine the dentary ridges covered with horn, then these, working against the tubercles would form a crushing or cutting mechanism, the mandibular edges acting as cutters and the tubercles keeping the food from slipping out of position. This implies some back and forth movement of the lower jaw. As Pearson (1924 b) and Watson (1948) have

pointed out, the structure of the condyle permits such movement, but whether it occurred or not would depend on the arrangement of the jaw muscles. Figure 3 shows what are believed to be the insertions of the muscles on the outer face of the mandible. The temporalis is attached to the highest point of the dentary and to the thickened ridge on its outer surface. From this

Fig. 7—Vertical sections through jaw margin ; A, near posterior end of anterior palatal ridges ; B, through external palatal tubercles ; C, through internal palatal tubercles.

point to its origin on the skull on the posterior and inner walls of the temporal cavity its fibres slope backwards. A roughened area on and directly above the reflected lamina of the angular appears to be the masseter insertion—a position suggested by Parrington (1955). If its origin was also as Parrington suggests, on the anterior part of the zygoma, then this muscle must have exerted a forward pull. There is a deep hollow where the articular overlaps the angular and there can be little doubt that the pterygoideus anterior wrapped round

the lower edge of the jaw to insert into this hollow. Its origin is marked by a slight roughening on the ventral and lateral faces of the anterior part of the pterygoid, just behind its junction with the ectopterygoid, which means that it also exerted a forward pull. The actions of the temporalis and masseter+pterygoideus anterior could therefore provide the requisite propalinal movement.

Three small bones, found lying loose in the temporal cavity of the right side, appear to belong to the hyoid apparatus. They consist of a triangular plate and two long thin bones, which are probably the median plate and the first and second branchial horns of one side (see Pl. 3 B). These structures are totally unlike the hyoid apparatus of snakes or varanids in which the long protusible vermiform tongue is primarily a sense organ used in combination with Jacobson's organ : they do, however, bear a considerable resemblance to the chelonian hyoid. It seems reasonable therefore to conclude that the tongue of *Daptocephalus* was relatively short and was used for manipulating the food in the mouth cavity. This is in accord with the fact that there is no opening from the palate to Jacobson's organ.

In connection with the masticatory apparatus one other structure remains to be considered. This is a fossa on the side of the face just behind the root of the tusk and below the anterior end of the zygoma : it will be referred to as the labial fossa (see Fig. 1). The labial fossa is a slightly bilobed pocket bordered below by the palatine and in front by the maxilla. Between these two bones the ectopterygoid just reaches the border of the fossa. Posteriorly a descending process from the jugal meets an ascending one from the palatine to form a pillar which bounds the fossa posteriorly but leaves a communication with the orbit mesial to the pillar. There is probably also a communication between the labial fossa and the deep cleft on the palate between vomer and palatine into which the choanae open anteriorly, but it is not possible to remove all the matrix in this area and make quite certain of this point.

The labial fossa is distinct from the foramen present in many reptiles, which Versluys (1936) calls the lateral palatal fenestra. This fenestra is present in *Kingoria* (Cox, 1959) and in *Cisticephalus* (Brink, 1950) and is thought to transmit the ramus of the trigeminal nerve which communicates with the palatine nerve. It lies internal to the ectopterygoid and is bounded by the latter bone, the maxilla and the palatine, whereas the labial fossa is external to the ectopterygoid and in contact with the jugal.

A labial fossa, similar to that of *Daptocephalus*, is figured by Toerien (1953) in *Lystrosaurus*, *Kannemeyeria* and *Aulacocephalodon*, but is absent in *Dicynodon* and the more primitive anomodonts which still retain post-canine teeth. It therefore seems to be a new structure, developed independently in a number of the more advanced forms and the problem of its function must therefore be considered. Its deep pocketed structure looks more like the housing of a reasonably large organ than a mere conduit for nerves or blood vessels and I suggest that it may have lodged a salivary gland.

In lizards and non-venomous snakes there is a strip of glandular tissue along the length of the upper lip (Bellairs 1957). This may be

assumed to be a primitive generalized condition. In venomous snakes the posterior part of this is concentrated and hypertrophied to form the poison gland. In *Daptocephalus* the chewing area lies far forward in the mouth, immediately behind the tusks ; a similar concentration of glandular tissue may have occurred at the anterior end of the strip, concentrating the secretion in the region where it is most needed. The bones give no clues as to what course the duct from the postulated gland may have taken.

THE AUDITORY REGION

The position of the fenestra ovalis in *Daptocephalus* is somewhat unusual (see Figs. 6 & 8). Instead of being directed sideways and slightly downwards it faces almost directly ventrally and lies at the base of an elongated bony tube projecting ventrally beneath the occipital condyle. The central portion of the tube is probably formed from the otic bones, although no sutures can be made out, but posteriorly it is formed from the basioccipital and anteriorly it is overlaid by a thin sheet of basisphenoid, continuous with the flat plate that covers the ventral surface of the pterygoids behind the inter-pterygoid fossa. The arrangement is essentially as described by Broom (1912) but in the species figured by him the basioccipital tubera are less elongated than in *Daptocephalus* and the fenestra ovalis faces more sideways and less downwards.

Specimen B.P.I. 349 has the right stapes *in situ*, with a little distortion of the region as a whole, the quadrate being probably displaced slightly inward and downward, so that the ventral slope of the stapes from its proximal to its distal end is slightly exaggerated. In addition, the distal end of the stapes appears to be a little crushed and distorted. The left quadrate is more crushed inwards than the right, and the stapes had become detached and lay in the back of the right temporal cavity close to the jaw articulation. It has been removed and completely cleaned so that all details of its structure are visible (see Pl. 5). The basic shape of the body of the bone is roughly what would result if a plate of plastic material, 3·5 cm. long by 3 cm. wide by 1 cm. thick were gripped by its two ends and these rotated through about 50° in opposite directions. Thus if one end is laid horizontally, the long axis of the other end will stand inclined at about 50°. Towards the proximal end of the shaft the ventral surface is scooped out into a deep groove. The structure of the faces at the two ends of the shaft shows the following complications. A slight groove runs along the long axis of the distal end. This articulates with a corresponding ridge on the inner surface of the quadrate just dorsal to the internal condyle. In addition the posterior corner is pulled out into a little flat plate making an obtuse angle with the quadrate articulation. The function of this will be discussed later. The proximal end is thickened up to form a triangle with its apex directed anteriorly, i.e. diagonally opposite the facet on the proximal end. The basal half of the triangle remains as a relatively flat surface, but the apex is built up to form a well-rounded articulating knob which, looked at from the proximal end, stands at a higher level than the remaining flat area. No stapedial foramen is present.

Owing to the crushing in of the quadrate, the left stapes cannot be replaced in its natural position in the skull. A cast of the distal end of the left occipital tuber with the fenestra ovalis has, however, been made. The articulating knob on the proximal end of the stapes makes an exact fit with the fenestra, the apex of the triangular articulation lies anteriorly and rises highest inside the tube, while the body of the stapes has only a slight downward slope towards its distal end. The facet on the corner of the distal end lies above the level of the internal quadrate condyle and faces outwards and slightly backwards.

While the lower jaw was being cleaned, a small bone was found lying loose against the mesial side of the right ramus just at the base of the retro-articular process. Since the skull shows lateral compression and the left stapes had fallen into the right temporal cavity, the skull must have been lying on its right side when it was fossilized. The little bone that had fallen down on to the right mandibular ramus must therefore have come from somewhere on the left side of the skull, nor far from the stapes. This had come to rest a little further forward, while the small bone's fall was stopped by the retro-articular process. The small bone has been removed and cleaned. It consists of a shaft, rounded rectangular in section approximately 10×6 mm. and 16 mm. in length (see Pl. 5). At one end it expands to form a little facet, slightly inclined to the axis of the shaft. If this facet is placed against the facet on the distal end of the stapes a good fit is obtained and I believe the little bone to be an ossified extra-stapes.

If this is correct, then its only possible function would be to connect the stapes with a tympanum. It therefore follows that *Daptocephalus* did possess a tympanum and that Watson (1953) is mistaken in supposing this structure to have been lost in the anomodonts : furthermore, since the distal end of the extra-stapes must have abutted on the tympanum, the position of the latter can be determined. In order to do this we must shift from the damaged left side of the skull to the undamaged right. Adding to the right stapes *in situ* an extra-stapes the same length as the left one brings one to a point directly above the internal quadrate condyle and below the edge of the ventral end of the squamosal. From this point a ridge, formed by the junction of the squamosal and opisthotic, runs up towards a blunt, backwardly directed projection of the opisthotic, the tympanic process of Cox (1959). The process is not nearly so long as the one described by Cox in *Kingoria*, but it could well form the upper attachment of an elongated tympanum which may be visualized as attached ventrally just above the inner condyle and anteriorly up along the ridge to the tympanic process (see Fig. 8). The latter lies mesial to the line of the condyle and the ridge slopes inward from the postulated ventral tympanal attachment in such a way as to keep the membrane clear of the retro-articular process as the jaw is opened. This arrangement is exactly as postulated by Cox, except that in *Daptocephalus* the shaft of the stapes lies almost at right angles to the basioccipital tuber. If the left hand extra-stapes, stapes and cast of the tuber are placed in their natural positions then pressure on the extra-stapes exerts a diagonal thrust on the stapes from the posterior corner to the articulation on the proximal end and presses the latter firmly against the fenestra ovalis. The whole would act as a sound conducting apparatus in

the classical manner, provided the tympanum was large enough to actuate the stapes. Hotton (1959) has carried out experiments with a model stapes and various sizes of tympanum. He expresses their relative sizes as the ratio of the volume of the bone (in mm^3) to the surface area of the membrane (in mm^2). In specimen B.P.I. 349 the volume of the stapes is almost exactly 10,000 mm^3 and a tympanic membrane of area approximately 450 mm^2 could have fitted in the position that has been suggested. This gives a bone : membrane ratio of 22 : 1. From his experiments Hotton concludes that for a large stapes the most effective bone : membrane ratio will be somewhere between 23 : 1 and 14 : 1. It therefore seems that the space available is enough to accommodate an effective tympanum. It must, however, be borne in mind that Hotton worked with a circular tympanum which was supported all round. In specimen B.P.I. 349 the tympanum, as restored, is elongated and cannot have been rigidly supported on its posterior border. Both these factors might make the membrane rather less effective than the figures given above suggest, but there does not seem to be any reason for concluding that such a tympanum would be functionless, or that the stapes did not conduct sound in the orthodox manner.

The position of the external auditory meatus and the external opening of the ear remain to be considered. On these points the bony structure of the specimen does not give any clear information, but it is difficult to see how the meatus could have been anything but a horizontal tube running immediately above the quadrate condyles to open just behind the angle of the jaw. The position of the depressor mandibulae muscle running up from the retroarticular process would seem to prohibit any other course for the meatus. Whether the action of the jaws in chewing would or would not cause some interference with hearing does not seem to be very important. I am indebted to Professor Bryan Patterson for making this point very effectively by the following comment : " If I want to hear properly what someone is saying while I am eating, I always stop chewing to listen ". No doubt *Daptocephalus* could have done the same.

THE OCCIPUT, CERVICAL VERTEBRAE AND NECK MUSCLES

As preserved, the occiput of specimen B.P.I. 349 consists of two expanded plates of bone, diverging laterally and meeting in the mid-line behind the parietal crest at an angle of about 45°, very much as shown for *Kannemeyeria* by Pearson (1924). As noted previously, there has certainly been some lateral compression and the lateral flanges of the occiput must have diverged more widely. In Fig 8 the occiput has been drawn as though completely flattened out into a transverse plate, since it is difficult otherwise to show the postulated muscle origins. It must, however, be realized that in reality the two sides lie at an angle to each other and the area above the foramen magnum is a deeply sunken groove running up to the posterior end of the parietal crest.

Just behind the parietal crest the upper wall of the occiput is formed by the postparietal, extending about half way to the point where the zygoma arises. From the end of the parietal the whole lateral surface of the occiput is formed by the squamosal, except for a narrow strip above the jaw

articulation where the quadratojugal is exposed to form the lateral border of the occiput.

The median portion of the occipital condyle is formed from the basi-occipital. This also forms the posterior walls of the ventrally directed tubera which have already been mentioned in connection with the auditory region. Laterally, the opisthotic lies between the condyle and the squamosal. The suture between opisthotic and squamosal can be traced from the lower border of the latter dorsally as far as the small posterior temporal fossa. The

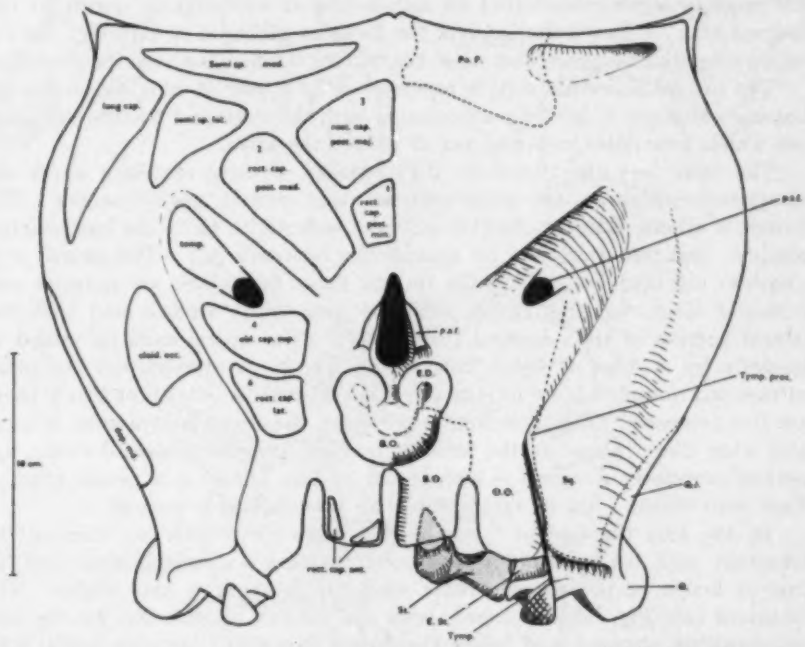

Fig. 8—Occiput, with muscle attachments shown on left. Cleid-occ., cleido-occipitalis muscle; dep. md., depressor mandibulae muscle; long. cap., longissimus capitis muscle; obl. cap. sup., obliquus capitis superior muscle; p.a.f., facet for pro-atlas articulation; p.t.f., post-temporal fossa; rect. cap. ant., rectus capitis anterior muscle; rect. cap. lat., rectus capitis lateralis muscle; rect. cap. post. maj., rectus capitis posterior major muscle; rect. cap. post. med., rectus capitis posterior medius muscle; rect. cap. post. min., rectus capitis posterior minor muscle; semi sp. lat., semispinalis capitis lateralis muscle; semi sp. med., semispinalis capitis medius muscle; temp., temporalis muscle; Tymp., tympanum; Tymp. proc., tympanic process of ophisthotic. (For abbreviations of names of bones see page 402.)

tympanic process of the opisthotic lies just mesial to this suture and about 4½ cm. above the ventral border of the squamosal. In the rest of the occiput the bones are closely fused and no sutures can be seen.

The post temporal fossa passes through the occiput with a downward slope from back to front: above the fossa there is a depression in which a small slip of the temporalis muscle almost certainly originated. On the mesial side

of this depression a pillar-like thickening of the bone slopes out and up from the border of the foramen magnum. This forms a strut strengthening the region from which the zygomatic wing of the squamosal is given off on the anterior face of the occipital plate.

The occipital condyle is tripartite, with the basioccipital forming the largest articulating surface. The condyle projects posteriorly well behind the occipital plate and the basioccipital forms a hemi-cylindrical downward facing articular surface above the tubera. The exoccipitals form the upper part of the condyle : their articular surfaces are round plates facing backwards and slightly sideways situated on either side of and slightly dorsal to the basioccipital. Above the condyle the foramen magnum is high and narrow with a roughly triangular facet on either side for articulation with the pro-atlas.

The pro-atlas (see Pl. 4 B) is represented by a pair of winglike structures bearing anteriorly a facet for articulation with the skull and postero-ventrally one which articulates with the neural arch of the atlas.

The atlas (see Fig. 9 and Pl. 5 F) consists of three elements which are completely unfused : the inter-centrum and paired neural arches. The former is a hemi-cylinder scooped out both anteriorly, to fit the basioccipital condyle, and posteriorly, to fit against the odontoid peg. The neural arch elements are complex. Ventrally on the inner face there are anterior and posterior facets for articulation with the exoccipital condyle and with the lateral portion of the odontoid respectively. The neural canal is walled in laterally by a ridge of bone, but is open above. Anteriorly on the outer surface is a rounded facet for the pro-atlas articulation and posteriorly there are two processes. The more ventral of these, the transverse process, is large and wing like : above it the smaller process projects postero-dorsally. A similar process in *Kingoria* is interpreted by Cox (1959) as a neural spine, a view with which I am in agreement. No atlantal rib is present.

In the axis the line of fusion of the atlas pleurocentrum, forming the odontoid, with the centrum of the second vertebra is clearly marked and the line of fusion of the neural arches with the centrum is also visible. The odontoid (see Fig. 10) is 3-lobed, with the median articulation for the axis intercentrum situated well below the lateral facets for the atlas neural arch elements. Between the two latter is a shallow median depression which probably marks the attachment of a retaining ligament whose anterior end was attached to the occipital condyle between the two occipital articulations. The neural arch (see Pl. 4 A) is expanded into a high dorsal flange, narrow for most of its length, but thickened anteriorly to form a flat surface directed upwards and forwards. The prezygapophyses are represented by small lateral flanges on either side of the neural canal : they cannot be brought into contact with the atlas neural arch elements, but probably were connected to the posterior end of the dorsal edge of the latter by ligaments. The posterior zygapophyses are well developed and articulate with the third vertebra in the normal manner. A large transverse process and a smaller apophysis low down on the side of the centrum articulate with the axis cervical rib. The latter is flattened and with a well-separated capitulum and tuberculum (see Pl. 4 C).

The third vertebra is much shorter from back to front than is the axis. It has not been possible to free it from the latter, so that the anterior face of the centrum is inaccessible. The posterior face is very deeply opisthocoelous, the depression reaching to just over half the thickness of the centrum. Only the bifid anterior end of the cervical rib is preserved.

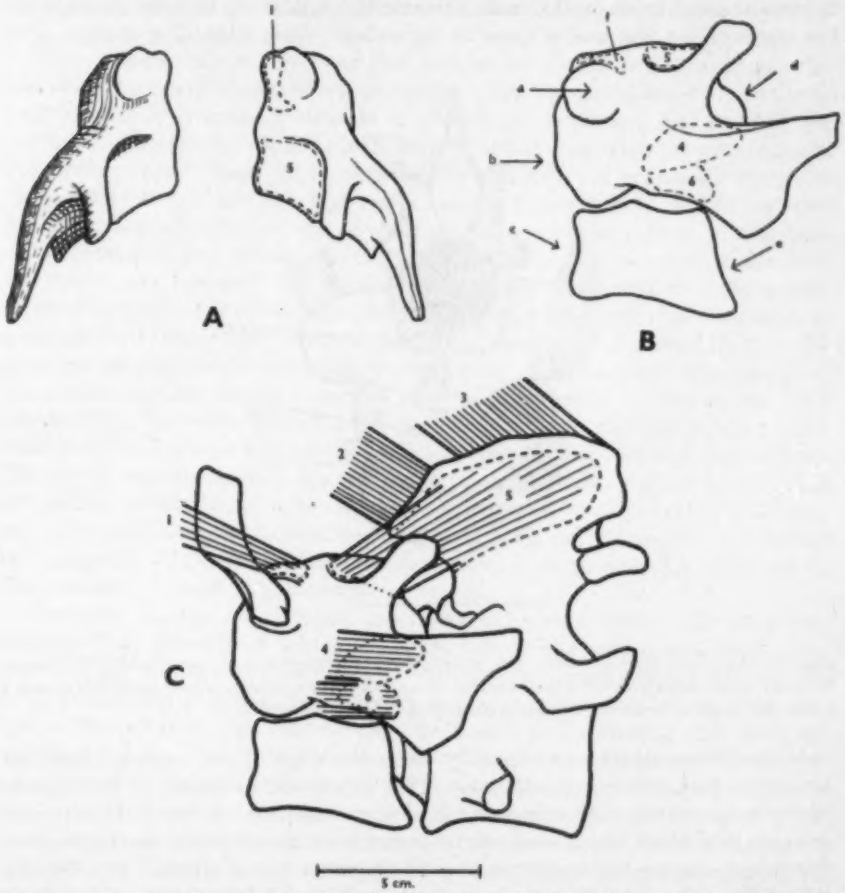

Fig. 9—A, atlas neural arches seen from above, muscle attachments shown on right ; B, atlas neural arch and intercentrum seen from left side ; C, pro-atlas, atlas and axis, side view to show muscles. 1–6 muscle attachments. 1, rectus capitis posterior minor ; 2, rectus capitis posterior medius ; 3, rectus capitis posterior major ; 4, obliquus capitis superior ; 5, obliquus capitis inferior ; 6, rectus capitis lateralis. a–e positions of articulating surfaces. a, with pro-atlas ; b, with exoccipital part of condyle ; c, with basioccipital part of condyle ; d, with lateral odontoid facet ; e, with ventral odontoid facet.

Before considering the neck muscles, it will be convenient to deal with those jaw muscles that originate on the occiput. Although the post-temporal fossa is small, its downward slope and the shape of the depression in the

occiput immediately above it suggest that a small slip of the temporalis passed through the fossa to attach on the occiput. The retro-articular process on the mandible, although short, is very stout and its roughened posterior end must have formed the insertion of the depressor mandibulae. In the living *Alligator* and *Varanus* the retro-articular process is long. In both, the origin of the depressor mandibulae on the skull starts on the side of the occiput close above the angle of the jaw and is there at its widest : from this point it runs as a

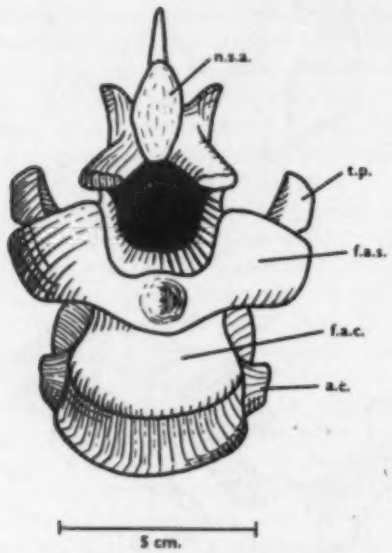

Fig. 10—Axis, seen from in front. a.c., apophysis for capitular articulation of axial rib ; f.a.c., facet for articulation with atlas intercentrum ; f.a.s., facet for articulation with atlas neural arch ; n.s.a., flattened anterior end of neural spine ; t.p., transverse process.

thin tendinous attachment dorsally along the edge of the occiput, reaching almost to the mid line in *Alligator*. The gradation in length of the muscle fibres is correlated with the position of their insertion on the retro-articular process : the fibres which insert distally and must therefore shorten most when the jaw opens are the longest ones with the most dorsal origin. In *Chelonia*, where the retro-articular process is short, and its end therefore does not move through a wide arc as the jaw opens, the muscle fibres are correspondingly short and the origin on the skull is not much extended dorsally. In specimen B.P.I. 349 a roughened area on the quadratojugal and squamosal where the two overlap on the occiput probably represents the origin of the depressor mandibulae. This is a normal reptilian position and is in keeping with the short retro-articular process. In *Kingoria* Cox (1959) believes that this muscle originated in a large fleshy attachment high on the occiput directly posterior to the base of the zygomatic ramus of the squamosal. This appears rather improbable. I know of no living reptile with anything resembling this

arrangement and it would produce a muscle with fibres much longer than are needed for the job they have to do.

Although muscle attachments are not clearly marked on the occiput, the structure of the atlas and axis allows one to make a reasonable restoration of the muscles attached to them while other neck muscles which are virtually constant in position in both reptiles and mammals may be assumed to have been present as usual.

The muscles which run from the occiput to the atlas and axis are the rectus capitis complex and obliquus capitis. The rectus capitis, both in living reptiles and in mammals consists of dorsal (or posterior) and ventral (or anterior) components, while in mammals a lateral component is also present. The rectus capitis posterior is variously subdivided : in mammals there are three parts, minor, medius, and major, inserted respectively on the anterior dorsal surface of the atlas, the anterior and the posterior regions of the crest of the enlarged axis neural spine. In reptiles there is less subdivision and the details are variable. In specimen B.P.I. 349 the flattened anterior end of the axis neural spine indicates the presence of a large rectus capitis medius, separate from the major, while a roughened area on the dorsal face of the atlas, postero-mesial to the proatlas articulation, indicates the attachment of the rectus capitis posterior minor. The rectus capitis posterior was thus divided into the three mamalian components and their attachments to the skull may therefore be assumed to be in the normal situations (see Figs. 8 & 9). The rectus capitis anterior muscles run from the ventral surface of the atlas and axis to attachments on the skull either below the condyle (as in *Alligator*) or, if the skull does not extend below the condyles, on the ventral surface (as in mammals). In *Daptocephalus* these muscles were presumably attached to the posterior faces of the basioccipital tubera.

In living reptiles the obliquus capitis muscle consists principally of fibres running from the side of the skull to the side of the axis neural arch, constituting the obliquus capitis magnus. In addition, some fibres run from the skull to the side of the atlas (obliquus capitis superior) and others from the side of the atlas to the side of the axis neural arch, forming the obliquus capitis inferior. The three components are not very clearly separated, the attachments of magnus and superior on the skull are confluent, as are those of superior and inferior on the atlas and of inferior and magnus on the axis. The three appear to function more or less as a unit and cause lateral bending of the head on the neck. It seems unlikely that this represents a primitive arrangement : more probably only the superior and inferior components were present originally, running in the normal manner between successive vertebrae and the separation of the obliquus capitis magnus as a more or less distinct muscle is a specialization which reflects the reduced size of the first vetebra and the unimportance of independent movement of atlas and axis. In mammals the obliquus inferior is very large and is responsible for rotating the atlas on the axis. The obliquus capitis magnus, which would interfere with this action, is missing and the obliquus capitis superior now inserts on the edge and lower surface of the wing-like lateral extension of the atlas. There does not seem to be any reason to assume that a separate obliquus capitis

magnus was ever present in the mammalian line, since it reflects the fact that independent atlas/axis movement is of little consequence, while in the mammalian lineage this movement becomes very important. In *Daptocephalus* the roughened dorsal surface of the atlas mesial to the neural spine must have formed the origin of the obliquus capitis inferior and the side of the expanded axis neural spine its insertion. Judging from the size of these areas, this muscle must have been large and rotation of atlas on axis well developed. The obliquus capitis superior, arising from the occiput below the posterior temporal fossa, must have inserted on the base of the atlas transverse process. To convert this to the mammalian condition, it is only necessary for the atlas neural spine to become united with the transverse process to form the lateral wing of the atlas, as suggested by Cox (1959). This suggested change is plausible, since the obliquus capitis inferior will work as an atlas rotator at a more advantageous angle if its attachment to the atlas is moved out laterally.

The mammalian rectus capitis lateralis was probably also present in *Daptocephalus*. There is ample space for its attachment to the skull between the occipital condyle and the tympanic process and a roughened area on the base of the atlas transverse process just ventral to the obliquus capitis superior attachment probably represents its atlantal insertion.

The neck muscles running from the occiput to the more posterior vertebrae are relatively constant in position and may therefore be assumed to have been present. These comprise the semispinalis capitis and the longissimus capitis. The former appears to have been divided into medius and lateralis, the medius originating in a depression high on the occiput between the posterior end of the parietal crest and the upper end of the zygomatic process of the squamosal and the lateralis ventro-laterally to this. On the lateral edge of the occiput the longissimus capitis would have originated lateral to the semispinalis lateralis. In addition, the trapezius presumably attached in its usual manner as a tendinous band along the upper edge of the occiput while a cleido-occipitalis lay mesial to the origin of the depressor mandibulae.

This restoration of the neck muscles agrees in general with Cox's restoration for *Kingoria* but there are a few points of difference. In *Kingoria* the axis spine gives no suggestion of subdivision of the part of the rectus capitis posterior which is attached to it : the latter muscle is therefore apparently made up of two components only, not three as in *Daptocephalus*. Such a difference is not surprising, since *Kingoria* is the more primitive. In addition, Cox considers that a large obliquus capitis magnus was present in *Kingoria*, whereas I do not think this muscle was present in the therapsids and we differ in our restoration of the origin of the depressor mandibulae. The latter two differences in interpretation should be regarded as such—not as established factual differences. In specimen B.P.I. 349 the muscle attachments on the occiput are not clearly marked and the restoration is largely a matter of deduction and inference, not of allotting particular muscles to clearly marked attachment areas.

THE MANUS

A number of the bones of the right manus are preserved (Pl. 5 F). These lay on the right side of the skull with their dorsal faces exposed. They are

considered to represent digits II to V, in each of which three phalanges seem to have been present. The two terminal phalanges are flattened and must have borne large, probably rather blunt, claws. The other phalanges and the three preserved metacarpals are short and the hand must have been broad and stumpy, very like the *Kannemeyeria* hand described by Pearson (1924 b).

In both *Lystrosaurus* and *Kannemeyeria* there are modifications of the phalanges which increase the degree of flexion of the digits, but the modifications are of two different kinds. In the *Lystrosaurus* hand described by Watson (1913) the first phalanx is peculiar in that the articulations slope inwards from above, so that the bone is triangular in longitudinal section. Although Watson, without giving any details, says that " the same feature occurs in *Kannemeyeria* " Pearson (1924 b) says that this is not the case in her specimen. There is, however, a feature which permits increased flexion of the terminal joint. The distal articulation of the second phalanx is not, as in *Lystrosaurus*, relatively flat and parallel to the proximal one, but curves down on to the ventral surface in such a way as to allow the ungual phalanx to be bent down almost through a right angle. In *Daptocephalus*, while the first phalanges have their articulating faces almost parallel, with at most a slight inward slope, the second phalanges have the same ventral prolongation of the articular surface as Pearson found in *Kannemeyeria*. It is not entirely clear whether the curious shape of the first phalanx in *Lystrosaurus* is related to aquatic habits but the *Kannemeyeria* second phalanx seems to reflect some use of the claws in scratching or digging, and the *Daptocephalus* foot is obviously of the same type.

FUNCTIONAL CONSIDERATIONS

In comparison with the less specialized anomodonts of the genus *Dicynodon*, the skull of *Daptocephalus* shows the following peculiarities.

(i) The basioccipital tubera are very long and are downwardly instead of laterally directed.

(ii) The skull is extremely high posteriorly and the postorbital region is long.

(iii) The temporal region is narrowed to a thin vertical crest.

(iv) The pterygoid extends far forwards to meet the maxilla and the ectopterygoid is correspondingly reduced.

(v) The posterior part of the roof of the mouth is extremely narrow.

All these features can be related to the propalinal jaw movement which the presence of the palatal tubercles and dentary ridges suggests must have occurred. In discussing the adaptations of carnivorous and herbivorous dinosaurs Colbert (1945) has pointed out that while a glenoid articulation on the same level as the tooth row is suited to a scissor-like cutting action, any form of crushing or grinding is much more effective when the articulation lies at a different level from the tooth row. The latter arrangement will be the more efficient for *Daptocephalus's* propalinal jaw action. In fact, the quadrate condyles do lie well below the level of the palatal tubercles. This position of the quadrate carries the distal end of the stapes down also and this, in turn,

necessitates prolongation of the basioccipital tubera in order to maintain the functional relations of the latter with the inner ear.

It has already been shown that the jaw muscles are arranged so that propalinal movement is achieved by the temporalis pulling the jaw backwards and the masseter and anterior pterygoideus pulling forwards. This has necessitated an insertion of the temporalis far forward on the dentary. This, in turn, means that the muscle fibres must be long so as to permit the jaw to open adequately. The requisite length is attained by increase in height of the posterior part of the skull, an adaptation paralleling the high posterior region of the skull in sabre-tooths. Since the brain is extremely small this increase in height occurs above the level of the braincase and there is therefore no reason to separate the sides of the skull by what would be an empty space above the brain. The intertemporal region is therefore narrowed posteriorly to form the typical crest.

In order to exert a forward pull, the masseter origin on the skull must lie well in advance of its insertion on the lower jaw : consequently the postorbital region of the skull must be long with the zygoma extending far forwards. Similarly, to give the anterior pterygoideus a strong forward pull, its insertion on the anterior end of the pterygoid must be carried forward, resulting in the anterior extension of this bone and corresponding reduction of the ectopterygoid.

The position of the chewing area relatively far forward just behind the tusks implies that the posterior part of the mouth has no masticatory function and is merely an anterior extension of the oesophagus. It is therefore not surprising to find that behind the chewing area the mouth becomes very narrow, which has the advantage of allowing more space for the jaw muscles. The latter must of necessity be powerful for the anterior position of the chewing area means that they work at a mechanical disadvantage.

In the neck region the atlas, axis and occipital condyles show differences from the typical reptilian condition which parallel the changes that occur in mammals. The significance of the changes is clear. In typical reptiles the body is low slung and the head is close to the ground. The movements which are important are up and down flexion and lateral bending, rotation being less important. Once the mammalian limb articulation is achieved and the body can be carried high off the ground, lateral flexion decreases in importance and rotation becomes essential if the side of the mouth is to be brought close to any object on the ground. The mammalian stance is therefore accompanied by specialization of the rotation of atlas on axis by means of the obliquus inferior muscle. Once rotation is established, quick lateral flexion of the head ceases to be important and this movement can be largely relegated to the neck, as distinct from the atlanto-occipital joint. The latter is now free to become specialized for the up and down movement which itself must become more extensive in correlation with the mammalian stance—as anyone who has watched a horse cropping grass can appreciate. It is therefore reasonable to expect that the changes correlated with rotation should precede those concerned with specialization of the up and down movement. The latter is achieved by the change to the mammalian double occipital condyle together

with obliteration of the atlas neural spine and increase in size and number of the rectus capitis posterior muscles. *Daptocephalus* shows the expected intermediate combination of characters : atlas/axis rotation is almost perfected but the atlas neural spine is still present as a separate process, although shifted laterally, while in the condyle the basioccipital articulation is still the largest, showing that specialization of the atlanto-occipital joint for up and down movement has not yet advanced very far.

ACKNOWLEDGMENTS

My thanks are due to the South African Council for Scientific and Industrial Research for a research grant, during the tenure of which part of this work was carried out. I should also like here to record my gratitude to the many honours students who have helped in this work ; both those who assisted in the initial stages of developing the specimen and those who, with unfailing cheerfulness, gave me help in coping with a specimen far too heavy to be managed single handed.

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ABBREVIATIONS OF NAMES OF BONES USED IN THE FIGURES

Ang.	Angular.	P. Mx.	Premaxilla.
Art.	Articular.	P.O.	Postorbital.
B.O.	Basioccipital.	Po. Fr.	Postfrontal.
Bs.	Basisphenoid.	Po.P.	Postparietal.
D.	Dentary.	P.P.	Preparietal.
Ecto.	Ectopterygoid.	Pre. Fr.	Prefrontal.
E.O.	Exoccipital.	Pt.	Pterygoid.
Epi.	Epipterygoid.	P. Sph.	Parasphenoid-basisphenoid.
E. St.	Extra-stapes.	Q.	Quadrate.
Fr.	Frontal.	Q.J.	Quadratojugal.
Ju.	Jugal.	S. Ang.	Surangular.
Lac.	Lacrimal.	S. Mx.	Septomaxilla.
Mx.	Maxilla.	Sp. Eth.	Sphenethmoid.
Na.	Nasal.	Sq.	Squamosal.
O.O.	Opisthotic.	St.	Stapes.
Pa.	Parietal.	Vo.	Vomer.
Pal.	Palatine.		

EXPLANATION OF THE PLATES

PLATE 1

Side view of the skull.

PLATE 2

A, mandible, side view. B, mandible, seen from above : on the left side the matrix has not been removed from the groove separating the two ridges on the posterior part of the dentary.

PLATE 3

A, Palate, showing palatal tubercles. The labial fossae can be seen on either side of the inter-pterygoid fossa. B, hyoid bones : scale in mm.

PLATE 4

A, axis and third vertebra, from right side : the axial rib is displaced ventrally. B, pro-atlas, ventral view. On the left of the photograph the facet for articulation with the skull can be seen on the external edge while on the right, the facet for articulation with the atlas lies at the end of the internal edge nearest the scale. C, axial rib of left side. All scales in mm.

PLATE 5

A & B, extra-stapes, posterior and anterior views ; facet for articulation with extra-stapes to the right. C, stapes, ventral view : facet for articulation with extra-stapes at top left. D, stapes, dorsal view : rounded surface for articulation with basioccipital tuber at top right, facet for articulation with extra-stapes at bottom left ; the deep groove between these two is not well shown. E, left atlas neural arch, from side. F, bones of right manus, digits 2 to 5, reading from right to left ; from dorsal surface except the second phalanx of the fourth digit, which has its ventral surface uppermost to show the rounded distal articulation. Scales in mm.

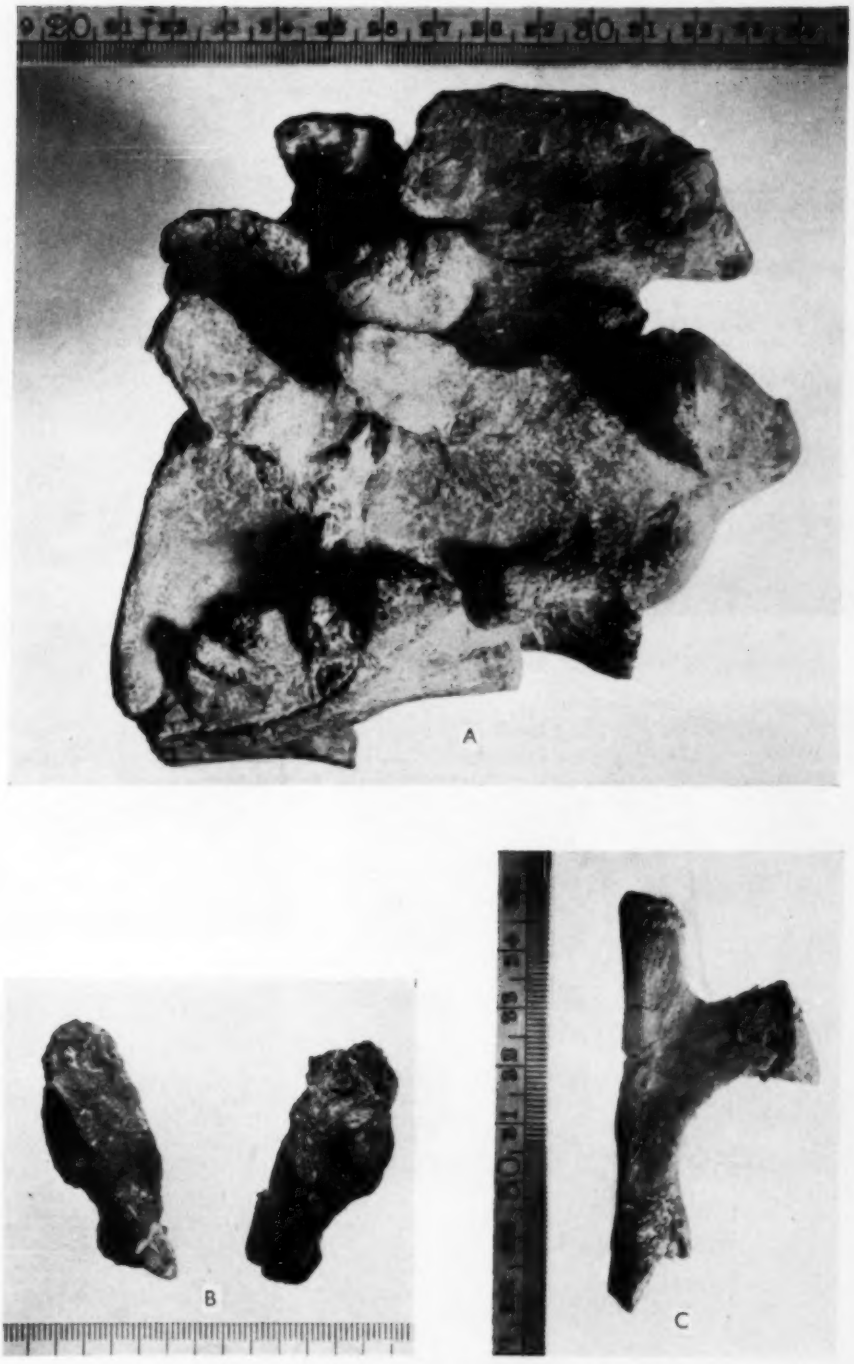
The anatomy of the anomodont *Deptocephalus leoniceps*.

A

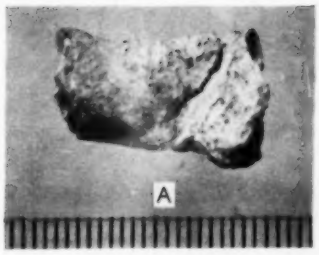
B

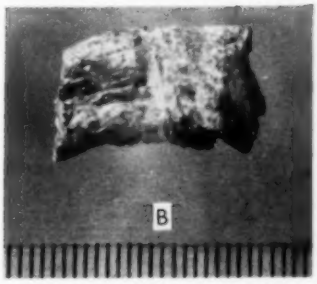
The anatomy of the anomodont *Deptocephalus leoniceps*.

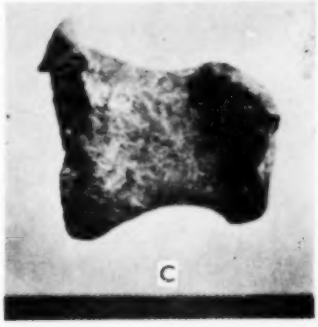
The anatomy of the anomodont *Deptocephalus leoniceps*.

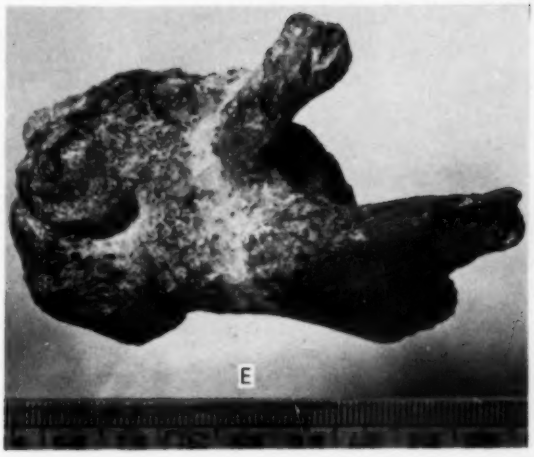


The anatomy of the anomodont *Deptocephalus leoniceps*.









The anatomy of the anomodont *Deptocephalus leoniceps*.

CONTINUOUS BREEDING IN THREE SPECIES OF TROPICAL ASCIDIAN

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(With 6 figures in the text)

The breeding season of three ascidians in Jamaica has been studied by counting the number of newly settled individuals each week on microscope slides suspended in the sea. Settlement occurs throughout the year with greatest numbers in autumn and early winter. These peaks cannot at present be correlated with any specific environmental influence.

CONTENTS

	Page
Introduction	403
Methods	404
Results	405
Discussion	408
Acknowledgments	409
References	409

INTRODUCTION

There is, at present, little information available on the breeding times of tropical marine animals. Over the past forty years a large body of information on the breeding times of temperate and high latitude forms has been accumulated, which supports the general conclusions of Orton (1914) that temperature is the dominant environmental influence controlling the breeding of such animals. Since temperature fluctuations appear to have a profound influence on breeding cycles it seems reasonable to suppose that greater insight into their fundamental causes may be obtained by a study of breeding cycles where temperature fluctuations are minimal, as in the tropics. In this paper the breeding cycles of three species of Jamaican ascidians are described.

Assessment of the breeding season of marine sessile animals can only be based on the presence of larvae in the plankton or of newly settled animals whose age can be determined with reasonable accuracy. The presence of sperm or ova in the genital ducts is not a suitable criterion as, even though these gametes are mature, they will only be shed under suitable environmental conditions. This viewpoint is strengthened by the findings of Sabbadin (1956) who records that *Botryllus schlosseri* in Venice has eggs in winter but that these are eventually resorbed without fertilisation. Furthermore, and for the same reason, the fact that an artificial fertilisation can be accomplished is not an indication that the animals are at that time breeding naturally. For this reason many statements in the literature about the breeding seasons of sessile animals are of little value.

As the larval life of ascidians is short (often less than twenty-four hours) it is not possible to determine the breeding season by taking regular plankton hauls; therefore reliance must be made on finding newly settled and metamorphosed individuals. Most previous studies on the breeding of ascidians and other fouling organisms have relied on the periodic examination (usually monthly) of test panels or tiles and determining the time when the young ascidians first appear. This method is valid as long as one knows something about the growth rate of the animal and can extrapolate to find the true spawning time. Thus *Ascidia nigra* is four to five weeks old before being visible to the naked eye and in colder climates growth of related species may be slower. An error may thus be created by regarding the appearance of young ascidians as a measure of breeding season and panels submerged for only four weeks will not necessarily have visible ascidians on it. Furthermore mortality is at its maximum in the first four weeks of life (own unpublished data for *A. nigra*) and the true peaks and troughs of breeding may be obscured by differential mortality.

For these reasons I have prefered to use microscope slides suspended in the water and examine them for newly settled animals at weekly intervals.

METHODS

Microscope slides were ground on one face with fine carborundum and mounted edge to edge in a perspex frame so that the ground surfaces presented a continuous flat settling surface of 320 sq. cm. The frame was backed by a piece of black perspex which held the slides in place and formed a dark background to the settling surface. Brass bolts held the two portions of the frame together and the frames were suspended in the water on galvanised chains. Two frames were suspended under the pier of the Royal Jamaica Yacht Club on the north shore of Kingston Harbour: all records for *Ascidia nigra* and *Symplegma viride* were obtained from here. A second pair of frames was suspended on either side of a raft moored at Morgan's Harbour, Port Royal, on the south side of the harbour. In both places the two frames were taken up each week and immediately replaced by a clean pair. The used frames were taken to the laboratory in sea water and the slides removed and examined under a binocular microscope. Magnification $\times 50$ was found to be suitable for identifying young ascidians.

Out of a large number of species of ascidians which settled on these slides only three could be identified in the early stages with sufficient certainty to be suitable for use in this study. *Diplosoma macdonaldi* Herdman is a colonial didemnid ascidian lacking spicules. It was found commonly on the Port Royal frames, but only rarely at the Yacht Club. All the records here are from Port Royal. *Ascidia nigra* (Savigny) is one of the commonest solitary ascidians in the harbour. The young animal is very transparent which distinguishes it from the rarer *A. interrupta* which is granular in the early stages. *Symplegma viride* Herdman is a colonial styelid and has a characteristic growth form producing stolonic vessels. In its very earliest stages it may be confused with a *Botryllus* but is more granular and careful inspection reveals lines demarcating the protostigmata which do not appear at this stage in *Botryllus*.

RESULTS

Figures 1 to 3 show histograms of the weekly settlement rate of the three species of ascidian over a period of two years. The dates at which records commence differ slightly for the three species and no observations were made

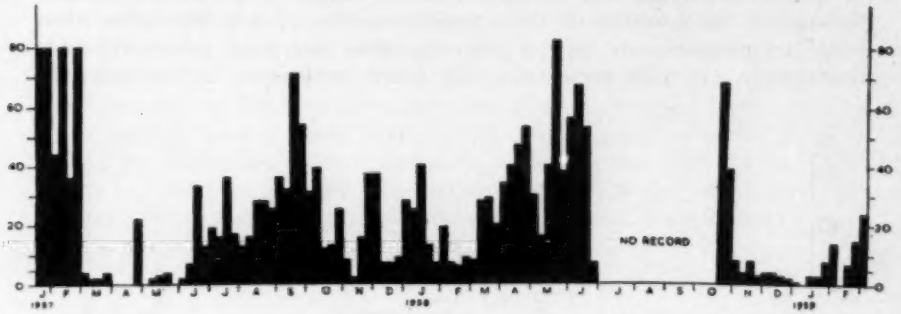

Fig. 1—Numbers of young *Diplosoma macdonaldi* settling on glass slides at Port Royal, Jamaica, in each week from January 1957 to February 1959.

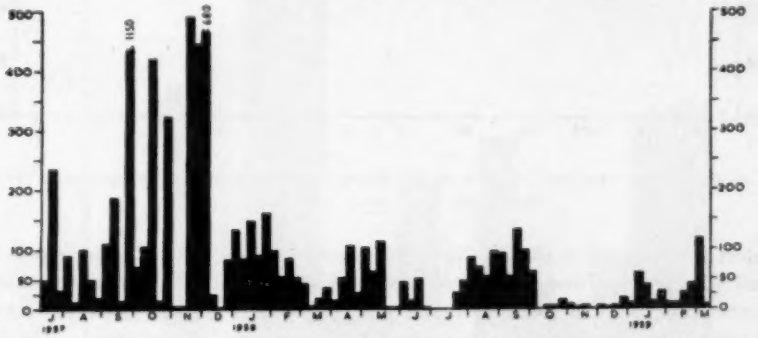

Fig. 2—Numbers of young *Ascidia nigra* settling on glass slides at Kingston, Jamaica, in each week from July 1957 to March 1959.

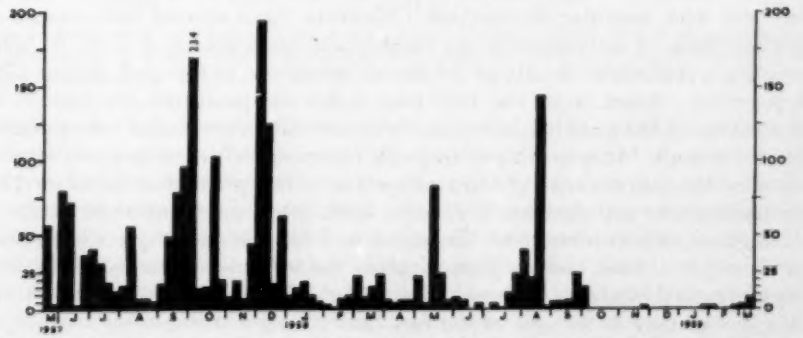

Fig. 3—Numbers of young *Symplegma viride* settling on glass slides at Kingston, Jamaica, in each week from May 1957 to March 1959.

on *D. macdonaldi* between the end of July and mid-October in 1958. All observations ceased in March 1959.

In all three species larvae have settled in every month of the year, but in no case is there an obvious pattern of reproduction. Numbers of *D. macdonaldi* fell off in 1957 from a peak in January to almost complete cessation of settlement throughout March and April, rising again to another peak in September when water temperatures are highest (Fig. 4); after this there are considerable fluctuations. In 1958 there were only a few settlements in February, the

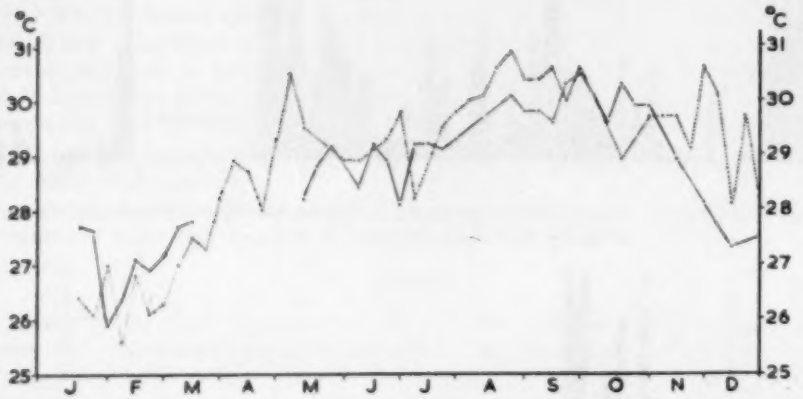

Fig. 4—Surface sea water temperatures at The Royal Jamaica Yacht Club, Kingston, in 1957 and 1958. 1957: ——— 1958: .....

coldest month, but unlike the previous year numbers were high in March and April through to June when they dropped again. It is unfortunate that no observations were made in August and September as the peak in October suggests that numbers may have been high again in late summer. The drop after October is discussed below.

The data for *A. nigra* and *S. viride* show continuous breeding throughout the year with irregular fluctuations. However, both species had a series of marked peaks of settlement in the autumn and early winter of 1957, *A. nigra* reaching a settlement density of 3·6 larvae per sq. cm. in the week ending 27th September. Apart from the fact that water temperatures are highest at this season of the year it is not possible to correlate these peaks with environmental stimuli. It is worth pointing out, however, that there is a relationship between the major peaks of the two species. The peak of *A. nigra* on 27th September was matched by *S. viride* a week later and so also the peaks of *A. nigra* on 19th October, 20th November and 6th December are all matched by *S. viride* a week later. This suggests the influence of some unidentified environmental stimulus at these times. The difference in timing between the two species may be related to the fact that *A. nigra* is oviparous with rapid development (sometimes settling as a larva within twenty-four hours of fertilisation of the egg—own unpublished observation) and *S. viride* is a viviparous

species with a developmental time of 140 hours at 16°C (Berrill, 1953): this time will probably be shorter at the higher temperatures encountered here.

Over the rest of the year the settlement rate of all three species, is probably entirely governed by the size of the adult breeding population in the vicinity at a given time, and no significance can be attached to the minor peaks and troughs.

The fall off in numbers in all three species in October 1958 is the direct result of a mass mortality of animals in Kingston Harbour following exceptionally heavy rain (Goodbody 1961). This killed ascidians in very large numbers and the breeding stock was consequently reduced. This precluded any possibility of the autumn peaks of 1957 being repeated in 1958. However data from another source are available for *A. nigra* to show how the settlement rate may vary from one month to another. At Port Royal, where the water is much deeper than at the Yacht Club and where for other reasons a larger

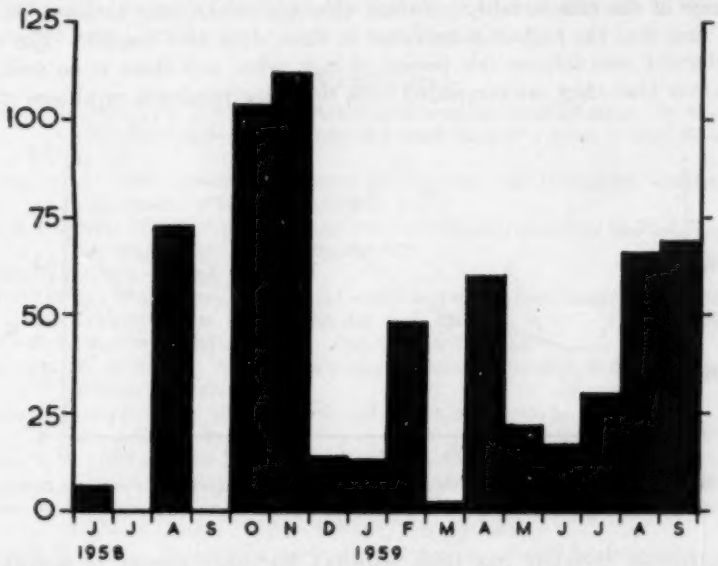

Fig. 5—Numbers of young *Ascidia nigra* settling on "Tuffnol" panels at Port Royal, Jamaica, in each month from June 1958 to September 1959 inclusive. No records were obtained in July and September 1958.

breeding stock survived, data have been collected on the monthly settlement rate on test panels of 1/8 inch thick Tuffnol measuring two feet square and suspended four feet below the surface. As *A. nigra* requires from four to five weeks to grow large enough to be seen with the naked eye each panel was left in the water for two months at a time: two panels were used and were raised and examined on alternate months. After counting the young ascidians the panel was scraped clean and replaced in the sea. The number of young *A. nigra* which settled on these are shown in Fig. 5. These figures substantiate the findings of 1957 that settlement is greatest in the autumn and early winter for this species.

DISCUSSION

The data outlined here show that these three species of ascidian breed continuously in the environment of Kingston Harbour, Jamaica, and suggest that peaks of settlement occur at certain times of the year. In *Diplosoma macdonaldi* the peaks are ill defined but in both *Ascidia nigra* and *Symplegma viride* there is a noticeable peak of settlement from September to the end of December. These fluctuations in settlement density may result from increased breeding activity on the part of the adults or from increased survival of eggs and larvae or a combination of both. No obvious correlation can be found between the peaks and any specific environmental factor. Surface sea water temperatures vary between about 25°C and 31°C and the maxima are reached in August and September so that the peak of settlement coincides with the beginning of a decline in surface temperatures. Tidal data (Fig. 6) show that the range of the tide is fairly constant throughout the year between 20 and 27 cm. and that the highest tides occur in June, July and August. The peak of settlement also follows this period of high tides, but there is no evidence whatsoever that they are correlated with tides, temperatures or phases of the moon.

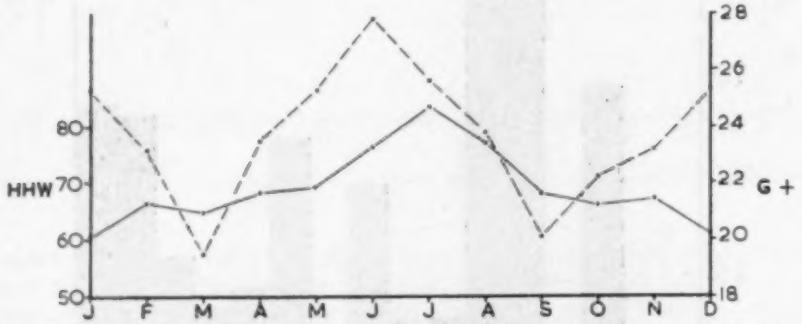

Fig. 6—Mean monthly high water (HHW: ————) and tidal range (G• – – – –) at Port Royal Jamaica in 1957. Measurements are in centimetres.

Continuous breeding has been recorded for other species of ascidian in other places but in many cases the conclusions are based on the presence of sperm or eggs in the genital ducts and are therefore an unreliable index of actual breeding. However, Skerman (1958) found that *Corella eumyota* settled all the year round at Lyttleton, New Zealand (43°S) where sea water temperatures vary between 7·2°C (July) and 19·9°C (January). Three other species were seasonal in the same locality. At Auckland (35°S) where temperatures vary between 11·3°C (August) and 23·1°C (February) the same author (1959) found *Diplosoma macdonaldi* and *Microcosmus kura* settling on panels throughout the year. Weiss (1948) found *Didemnum candidum* settling in every month of the year in Biscayne Bay, Florida (25°N) but two other species were seasonal. Smith *et. al.* (1947) record that in the inshore waters adjacent to Miami, Florida (25°N) ascidians settled throughout the year but they do not give separate data for each species. In general, however, it seems that

outside the tropics, ascidians are seasonal in their breeding activity: data in support of this may be found in Allen & Wood (1950), Brewin (1956), Kott (1952), Marine Biological Association (1957), McDougall (1943), Millar (1952, 1954 a, 1954 b, 1958), Orton (1914, 1920), Sabbadin (1956, 1957) and Skerman (1958, 1959).

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Grateful acknowledgment is made to the Nuffield Foundation from whom the author was in receipt of a research grant when these observations were made. Also to the Director, U.S. Coast and Geodetic Survey for permission to reproduce the data in Fig. 6. I am also most grateful to my wife who undertook the routine examination of slides from the Yacht Club in 1958 and 1959 and to Professor D. M. Steven who examined the Tuffnol panels at Port Royal (Fig. 5) during my absence from the island in the summer of 1959. Acknowledgment is also made to the Secretary and Committee of the Royal Jamaica Yacht Club for permission to use their pier.

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THE POSTEMBRYONIC DEVELOPMENT OF THE FEMALE REPRODUCTIVE SYSTEM IN *LIMOTHRIPS CEREALIUM* HALIDAY (THYSANOPTERA : THRIPIDAE)

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(With 14 figures in the text)

In the early second-instar larva the reproductive system comprises a pair of undifferentiated ovary-rudiments, each produced posteriorly into a solid strand of cells—the mesodermal lateral oviduct rudiments. Later in the second instar a spermathecal rudiment arises from the epidermis of the eighth abdominal segment and ovipositor valve rudiments from the eighth and ninth sterna. By the early prepupa the lateral oviducts acquire ampullae and the ectodermal structures are supplemented by a common oviduct rudiment (from the seventh sternum) and an accessory gland rudiment (from the ninth sternum). Further growth of these is followed in the pupa by the development of the vagina through ventral closure of a mid-sternal groove on the eighth segment and by the division of the ovary rudiment into ovarioles. Meanwhile the ovipositor valves have increased in length and some of their muscles have arisen by differentiation of previously unorganized masses of myoblasts ; the remaining ovipositor muscles are formed from larval muscles which change their positions but are otherwise unaltered. Though the reproductive system of *Limothrips* is morphologically comparable to that of less specialized Exopterygota, its development is shown to be delayed in a manner more reminiscent of the endopterygote insects.

CONTENTS

	Page
Introduction	411
Interpretation of adult system	412
Second-instar larva	413
Prepupa and pharate pupa	417
Pupa and pharate adult	423
Mature adult	430
Discussion	432
Summary	435
References	435

INTRODUCTION

It is well known that the Thysanoptera have an anomalous type of metamorphosis in which two larval stages are followed by a prepupa and one or two pupal instars before the adult is finally reached. Despite the great general interest of this peculiar life-cycle, little is known of the histological changes which occur during postembryonic development (Müller, 1927 ; Melis, 1935 b). In particular, only the most incomplete information is available on the development of the ovaries, efferent duct system and ovipositor of the terebrantian families. Jordan (1888), in the first adequate account of the internal anatomy of several species, found that the rudiments of the four ovipositor valves arise in an early larval instar, of which he mistakenly thought there were four.

One pair of rudiments arises from the epidermis of the eighth abdominal segment, the other pair from the ninth. Jordan also described the median infolding of the eighth to tenth sterna, noted the contributions made by the eighth and ninth sterna to the basal structures of the ovipositor, and mentioned that the rudiment of the vagina arises between the bases of the developing valve rudiments. Müller (1927) observed the differentiation of the ovarioles from an undivided ovary rudiment during the pupal stage of *Parthenothrips dracaenae* (Heeger), a feature further described in the tubuliferan *Liothrips oleae* Costa by Melis (1935 b). The latter author also recorded the presence of lateral oviduct rudiments and, in the prepupa, of an S-shaped epidermal invagination occupying the seventh to ninth abdominal segments and representing an early stage in the development of the ectodermal parts of the efferent duct system.

Clearly, these scanty data are quite insufficient to allow anything approaching a clear understanding of the development of the female reproductive system of the Thysanoptera. The following account of *Limothrips cerealium* Haliday—whose general biology is dealt with by Sharga (1933 a) and Lewis (1959)—is based on a collection of serial sections which cover the period from the second larval instar to the mature adult. In all, eighteen different stages were represented in the material so that an almost continuous picture of the developmental changes was obtained. Freshly-killed material was fixed in Duboscq-Brasil, prepared for sectioning by a slight modification of Péterfi's (1921) method of celloidin impregnation, cut at 5 or 6 μ and stained with Ehrlich's haematoxylin and eosin. Selected transverse sections are illustrated below and have been supplemented by diagrammatic reconstructions of the developing genital area. These were prepared by plotting on graph paper the positions of suitable landmarks from each successive transverse section of a series. They are therefore tolerably accurate scale reproductions of the organs concerned, though it must be remembered that individual specimens of the insect differ somewhat in size and, to some extent, in the precise shape and relative position of the developing structures. Undue significance should therefore not be attached to minor differences between the different stages.

INTERPRETATION OF ADULT SYSTEM

Before describing the processes of postembryonic development it is necessary to be clear as to the anatomy of the adult female terebrantian (cf. Figs. 13 & 14). This has not been very fully investigated and there are several discrepancies between the various published accounts. Cary (1902) claimed that *Anaphothrips striata* Osborn had five ovarioles in each ovary, but all the other investigated species, including *Limothrips cerealium*, have only four (Jordan, 1888 ; Buffa, 1898 ; Klocke, 1926 ; Sharga, 1933 b ; Lewis, 1959). From each ovary there runs a short lateral oviduct which unites with its fellow in the mid-line to continue back as the common oviduct and/or vagina, no clear distinction having been drawn between the latter two regions. The main disagreement concerns the annexes to the reproductive tract. Cary (1902) could find neither spermatheca nor accessory glands in *Anaphothrips striata*, and Buffa (1898) reported no accessory gland in *Heliothrips haemorrhoidalis* (Bouché). But

other accounts usually refer to two structures : a relatively small brownish body close to the vagina and opening at about half way along its course, and a much larger retort-shaped organ with a relatively long duct opening more posteriorly. Both these were easily identified in my sections of *Limothrips*. Jordan (1888) regarded the small ' brown body ' as a lubricating gland and the retort-shaped structure as the spermatheca, though he apparently did not investigate the presence of spermatozoa in any of the Terebrantia which he discusses. Buffa (1898) figures and describes as the spermatheca of *Heliothrips haemorrhoidalis* what evidently corresponds to the brown body but found no spermatozoa in it as the species is normally parthenogenetic. Klocke (1926), who worked mainly on sections of *Thrips physapus* L. and *Aeolothrips fasciatus* (L.) asserts categorically that the brown body is the spermatheca and the retort-shaped organ an accessory gland. He could never find spermatozoa in the retort-shaped organ, but unfortunately he does not say whether they were present in the brown body. Pussard-Radulesco (1931), who claims to have found spermatozoa in females of *Limothrips denticornis* Haliday, did not identify the organ in which they occurred beyond saying it was the spermatheca. Finally Sharga (1933 b), who dissected *Melanthrips fuscus* (Sulzer) and eight species of Thripidae, identified the retort-shaped organ as the spermatheca (allegedly containing a ' dense collection of sperms '—though it is not clear whether these were observed in the Terebrantia or only in a tubuliferan which he also dissected). He omits to discuss the function of the brown body.

Some of the uncertainty has perhaps arisen through attempts at equating the terebrantian organs with the rather different tubuliferan ones (cf. Melis, 1935 a). At all events, the situation remains confused in all general accounts of the order. Priesner (1928) and Morison (1947–49) follow Jordan's and Sharga's interpretation, while Pesson (1951) summarizes the differences of opinion without settling anything. Only Heberdey (1931) accepts Klocke's view, though on what grounds is not evident. My observations on *Limothrips cerealium* and also on *Odontothrips ulicis* (Haliday) leave no doubt that Klocke was correct. Spermatozoa are present in the small brown body (Fig. 14 B) and never in the retort-shaped organ. The latter contains a copious secretion and is undoubtedly an accessory gland of some kind. This conclusion agrees with two other findings : the epithelium of the retort-shaped organ is obviously glandular (Figs. 14 E, F, and p. 431) while that of the brown body is not; and the site of origin of the two structures, as shown below, accords with what is known of the development of the spermatheca and accessory glands in most other insects. In what follows, therefore, the terms spermatheca and accessory gland are used for the brown body and the retort-shaped organ respectively. Other aspects of the adult system are discussed later (p. 430).

SECOND-INSTAR LARVA

Three successively older stages were studied in the second instar. They show the origins and progressive growth of the ovipositor valve rudiments, but also demonstrate that little development of the ovary and efferent duct system occurs during this period of larval life.

The ovary rudiments are small, broadly pyriform structures with the blunt end anteriorly situated. They are 40–45 μ long, have a maximum transverse diameter of about 30–35 μ and lie just below the dorsal epidermis of the fifth abdominal segment, though in some specimens they may project into the fourth or sixth segments for a short distance. Each ovary is partly enclosed

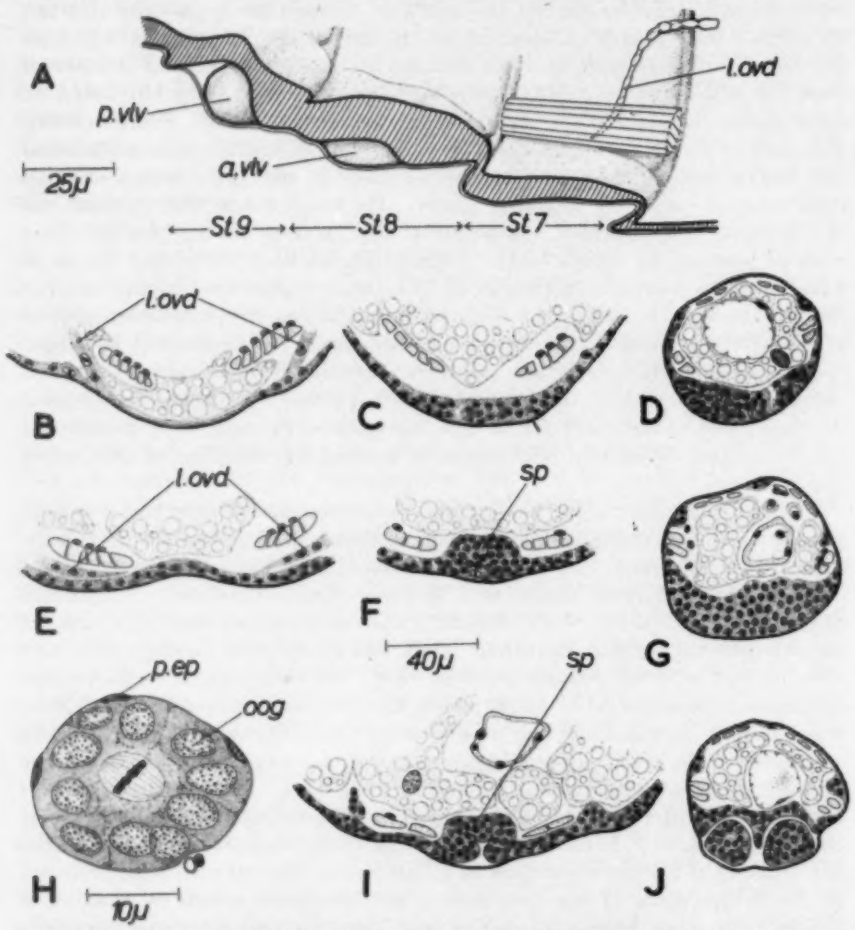

Fig. 1—Female genital region of second-instar larva. A. Reconstruction of older larva, the approximate segmental boundaries indicated. B. Seventh sternum and lateral oviduct rudiments of early stage. C. Eighth sternum of early stage. D. Ninth sternum of early stage. E. Seventh sternum and lateral oviduct rudiments of late stage. F, I. Eighth sternum of late stage, F anterior to I, the latter showing anterior valve rudiments as lips of median groove. G, J. Ninth sternum of late stage, G anterior to J, the latter showing posterior valve rudiments ending freely. H. T.S. ovary rudiment towards anterior end, showing oogonial mitosis.
a.vlv, anterior valve rudiment ; *l.ovd*, lateral oviduct rudiment ; *oog*, oogonium ; *p.ep*, primary epithelial sheath ; *p.vlv*, posterior valve rudiment ; *sp*, site of spermathecal rudiment (hardly differentiated) ; *St.* 7, 8, 9, limits of seventh, eighth and ninth abdominal sterna.

by a lobe of the fat-body and almost touches its fellow of the opposite side. There is no sign of a division of the rudiment into ovarioles and no indications of a terminal filament. Posteriorly the rudiment narrows abruptly into the rudiment of the lateral oviduct.

The histological structure of the ovaries is very simple at this stage (Fig. 1 H). The whole organ is enclosed in a very thin squamous epithelium corresponding to the primary epithelial sheath of Wick & Bonhag (1955). This is only 1–2 μ thick with a few flattened and strongly basiphil nuclei measuring 5–6 μ in their long axis. Fine tracheal branches run close to or are apposed to the outer surface of the sheath. Within it is a mass of densely packed, irregularly arranged oogonia, the only other type of cell present in the ovary at this stage. The oogonia (Bonhag 1958), whose boundaries are not always very distinct, are irregularly shaped, vary from 5 to 7 μ in diameter, and contain conspicuous ovoidal nuclei of about 5·5 by 4 μ (Fig. 1 H). Their cytoplasm, in preparations fixed with Duboscq-Brasil, is uniform and finely granular, somewhat eosinophil and without any obvious cell-inclusions. It varies in amount, some nuclei being associated with an appreciable bulk while others are enclosed in only a thin film. The nuclei are rather weakly basiphil, tending to approach a vesicular appearance in older larvae, with small irregular chromatin masses somewhat more concentrated peripherally and without a distinct nucleolus. At this stage the oogonial nuclei are much larger than those of the epidermis, muscles or central nervous system (the smallest nuclei in the body, measuring 3–4 by 2 μ) but appreciably smaller than those of the fat-body or mid-gut epithelium (8–9 by 6–7 μ). Occasional mitotic figures are to be seen among the oogonia (Fig. 1 H), apparently confined to or predominating in the anterior half of the ovary rudiment. Otherwise the only suggestion of different forms of oogonia is a slight tendency for the larger ones to occur posteriorly, though the maximum nuclear size observed did not exceed 8 by 6 μ. Estimates of the number of oogonia were made by counting the numbers of nuclei in alternate sections ; there are about 60–100 in each ovary.

Sections through the extreme posterior region of the ovary show no oogonia, but the primary epithelial sheath cells are less flattened here and more prominent nuclei, still retaining their densely basiphil character, are now to be seen. These cells, which are radially arranged around a region of less dense cytoplasm or, in the oldest larva, around a small but patent lumen, pass continuously into the cells of the lateral oviduct rudiment. This consists of a very thin solid strand of cells which, on leaving the ovary, first runs postero-laterally. Then, entering the fat-body, it bends more abruptly ventralwards at the back of the sixth abdominal segment. Finally, in the posterior third of the seventh segment it turns medially and ends there in a delicate cytoplasmic attachment to the epidermis immediately below the more lateral of the ventral longitudinal muscle fibres (Figs. 1 B & E). The diameter of this strand rarely exceeds about 3 μ and it consists of a row of small, strongly basiphil nuclei with a thin film of cytoplasm. The nuclei are mostly arranged one immediately behind the other, though occasionally two or even three occur side by side. The lateral oviduct does not develop appreciably in the second instar, but occasional mitoses are visible at its posterior end in the two older

stages. There are no genital ampullae associated with it though, as will be shown below, these are present in the earliest prepupa examined and may well have developed at the end of larval life or in the pharate prepupa.

The ovipositor and ectodermal components of the reproductive system arise from a more or less confluent group of bilaterally symmetrical genital discs which are derived from the epidermis of the seventh, eighth and ninth abdominal sterna and whose origin and development during the second larval instar must now be traced (Fig. 1). During the early part of the stadium the epidermis of these sterna is in no way unusual, consisting of a normal single layer of cells, averaging about 3 μ in thickness and with well-separated and only slightly basiphil nuclei. The first indications of development are seen in occasional mitotic figures with some hypertrophy and closer nuclear aggregation at the back of the ninth segment. These processes then continue so that the epidermis of the posterior third of the seventh sternum thickens to about 6–10 μ with nuclei occasionally lying one on top of the other and an increase in basiphil staining (Fig. 1 E). Meanwhile the epidermis of the eighth sternum has also become hypertrophied over its posterior two-thirds (Fig. 1 C), and begins to acquire a more elaborate structure. The anterior part of the disc thus becomes differentiated into a median proliferation about 20 μ thick and 30 μ broad (Fig. 1 F), with a thinner lateral zone whose margins are again locally thickened and above which lie the longitudinal ventral muscles of the eighth segment. Posteriorly the median proliferation passes into a pair of slightly divergent lips flanking a median longitudinal groove (Fig. 1 I). The lips will eventually expand into the anterior valves of the ovipositor while the median groove and anterior median proliferation indicate the site of origin of the spermatheca. The whole disc now contains tightly packed, densely basiphil nuclei, several layers thick in the more highly proliferated regions and one or two layers thick elsewhere with nuclear division still in progress. At the same time the epidermis of the ninth sternum undergoes extensive hyperplasia to a thickness of about 12 μ anteriorly and 18 μ posteriorly (Figs. 1 D & G) while still further back the disc is organized into a pair of lateroventral lobes which project freely towards the posterior margin of the segment and represent the posterior ovipositor valves (Fig. 1 J). The epidermis of the anterior part of the tenth sternum also undergoes some thickening during the second stadium ; this is of no great morphological significance, but allows for the development of the deep median infolding which will eventually accommodate the more fully developed ovipositor in the ensuing instars.

Thus, the general appearance of the genital area in the oldest second-instar larva examined is shown in Fig. 1 A. At this stage the sternal boundaries are still clearly defined by the larval musculature, the early rudiments of the two pairs of ovipositor valves are apparent, and there is some indication of an internal ingrowth of epidermal cells on the eighth sternum which will eventually form the spermatheca. But the development of the common oviduct and accessory gland rudiments (from the discs of the seventh and ninth segments respectively) has hardly begun. They perhaps differentiate at the very end of the instar or in the pharate prepupa, which was unfortunately not represented in the available series of stages. Certainly in the earliest prepupa examined they are clearly defined.

PREPUPA AND PHARATE PUPA

The prepupa and pharate pupa together occupy an appreciably shorter part of the whole life-cycle than do any of the other instars (Sharga 1933 a). It is therefore not surprising that the developmental changes in the reproductive system are then less extensive than those which will take place in the pupa. Nevertheless some important advances may be seen.

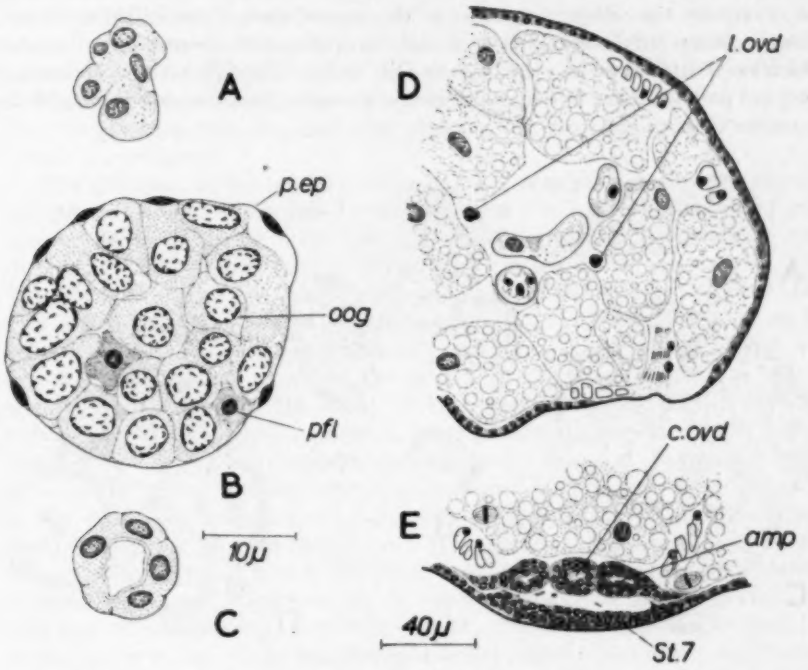

Fig. 2—Mesodermal parts of developing female reproductive system in young prepupa. A. Transverse section of common apical filament. B. Transverse section of ovary rudiment at about middle. C. Transverse section at junction of ovary and lateral oviduct rudiment. D. Transverse section of abdomen near boundary between fifth and sixth segments, showing appearance of lateral oviduct rudiments. E. Transverse section through genital ampullae, common oviduct rudiment and seventh sternum.

amp, genital ampulla ; *c.ovd*, common oviduct rudiment ; *l.ovd*, lateral oviduct rudiment ; *oog*, oogonia ; *p.ep*, primary epithelial sheath ; *pfl*, prefollicular cell ; *St.* 7, seventh abdominal sternum.

By the early prepupa the ovary rudiment has come to differ a little from the condition described in the second-instar larva. Its main bulk is now broadly ovoidal rather than pyriform and though still not subdivided into ovarioles the anterior region is produced into a terminal filament which, soon after its origin, is fused with that of the opposite side. The common filament thus formed runs forwards into the fourth abdominal segment where it seems to end in contact with a lobe of the fat-body. It is composed of scattered nuclei measuring about 5 by 3 μ, arranged irregularly in a homogeneous

cytoplasmic matrix and apparently without a surrounding epithelial sheath Fig. 2 A). The oogonia of the main ovoidal part of the ovary (Fig. 2 B) are still closely and irregularly packed together with a few mitoses visible anteriorly, but some growth has occurred in the more posterior ones. Anteriorly the oogonial nuclei have a long diameter of about 6 μ and possess more abundant and more uniformly distributed chromatin ; posteriorly they may attain 9 μ in length and are rather more vesicular in appearance. The main advance in the ovary at this stage, however, is the appearance of prefollicular tissue. This comprises small cells with uniformly basiphil nuclei about 3 μ in diameter which are scattered among the oogonia (Fig. 2 B). Though not very numerous they are perhaps more frequent anteriorly, though it has not proved possible to ascertain their origin.

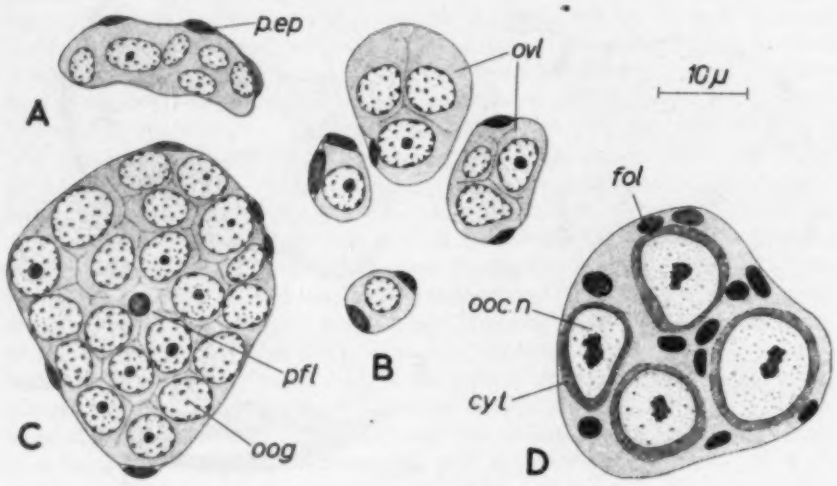

Fig. 3—Developing ovary of pharate pupa. A. Common terminal filament. B. Anterior part of ovary showing division into four ovarioles. C. Middle part of ovary, undivided. D. Basal region of ovary, undivided but with maturing oocytes and incipient follicular tissue.
cyt, cytoplasm of oocyte ; *fol*, follicular cells ; *ooc.n*, nucleus of oocyte ; *oog*, oogonium ; *ovl*, ovariole ; *p.ep*, primary epithelial sheath ; *pfl*, prefollicular cell.

The changes which occur in the ovary during the prepupa may be appreciated by comparing the above situation with that in the pharate pupa (Fig. 3). The ovary increases in length by about fifty per cent, exclusive of the common anterior filament, and the latter comes to assume a more organized appearance with its posterior region composed of small oogonium-like cells enclosed by an epithelial layer (Fig. 3 A). The anterior third or so of each ovary may also begin to show division into four ovarioles at this stage (Fig. 3 B) though in other specimens this process is deferred until the pupa. In any case, for most of its length the ovary remains undivided (Figs. 3 C & D) though it has increased somewhat in diameter to a maximum of 40 μ. Histologically there is a pronounced differentiation of the older, more posterior, germ-cells which

may now be regarded as primary oocytes (Fig. 3 D). These have grown to about 15–17 μ across with nuclei of 12–14 μ. The oocyte nuclei are clearly vesicular with a peripheral layer of granular chromatin and a somewhat diffuse central or slightly excentric nucleolus, both rather strongly basiphil. The homogeneous and also somewhat basiphil cytoplasm is still relatively scanty, however, and the oocytes are still irregularly arranged (the fact that four occur in Fig. 3 D is fortuitous). Among the primary oocytes in a uniform cytoplasmic matrix are small strongly basiphil nuclei which, since they are now beginning to arrange themselves around the oocytes, may perhaps be regarded as constituents of follicular rather than prefollicular tissue (Fig. 3 D). Mitoses continue to occur among the more anteriorly placed oogonia and the total number of germ-cells (oogonia plus primary oocytes) has now increased to about 120 per ovary.

The rudiment of the lateral oviduct changes very little during the prepupa and pharate pupa. It remains for the most part a delicate solid strand of cells, usually about 3 μ in diameter but occasionally enlarging to about 6 μ, with one, two or three densely basiphil nuclei visible in transverse section (Fig. 2 D). Posteriorly, however, the lateral oviducts have, by the earliest prepupal stage seen, expanded into a pair of genital ampullae (Figs. 2 E and 4 A). These are solid pear-shaped structures containing small irregularly packed nuclei, and are closely associated at about the middle of the seventh sternum with a median ingrowth from the back of that segment (the common oviduct rudiment). A few mitotic divisions can be seen among the nuclei of the ampulla, but it only develops the beginnings of a lumen in the pharate pupa. It is perhaps worth making clear that though I have regarded the ampullae as the posterior terminations of the mesodermal lateral oviduct rudiments there is no direct proof of this in the Thysanoptera ; their origin between the second-instar larva and early prepupa could not be traced in the material available and my interpretation is based mainly on analogy with the orthopteroid insects (e.g. Nel, 1930 ; Qadri, 1940). The possibility that the ampullae are really of epidermal origin and acquire a secondary association with the strand-like lateral oviduct rudiments—as Heberdey (1931) claims for some Coleoptera and Abul-Nasr (1950) for some Diptera—requires further investigation, though the appearance and relations of the newly-formed ampullae tend to support the older view.

By the early prepupa the genital discs of the seventh, eighth and ninth abdominal sterna have assumed a structure which begins to foreshadow the adult condition more clearly (Fig. 4 A). At this stage and throughout the rest of the pre-imaginal period most of the ventral epidermis is strongly basiphil, much thickened, and the separate discs are now barely, if at all, distinguishable, while the boundaries of the sterna become progressively less distinct. At the back of the seventh segment there is a thick-walled invagination provided with a small but distinct lumen and projecting forwards between the ampullae (Figs. 2 E & 4 B). This is the rudiment of the common oviduct and its subsequent development up to the pharate pupa comprises some increase in size, slight growth of its lumen, further detachment from the epidermis of the seventh sternum proper, and a more intimate association with the ampullae

(Figs. 5, 6 A & B), while its posterior opening becomes extended into a short median groove (Fig. 6 C). Behind this opening lies the strongly thickened epidermis of the eighth abdominal sternum, which is reflected back at about its middle to form a cavity (seen in cross-section in Fig. 4 D). The lateral

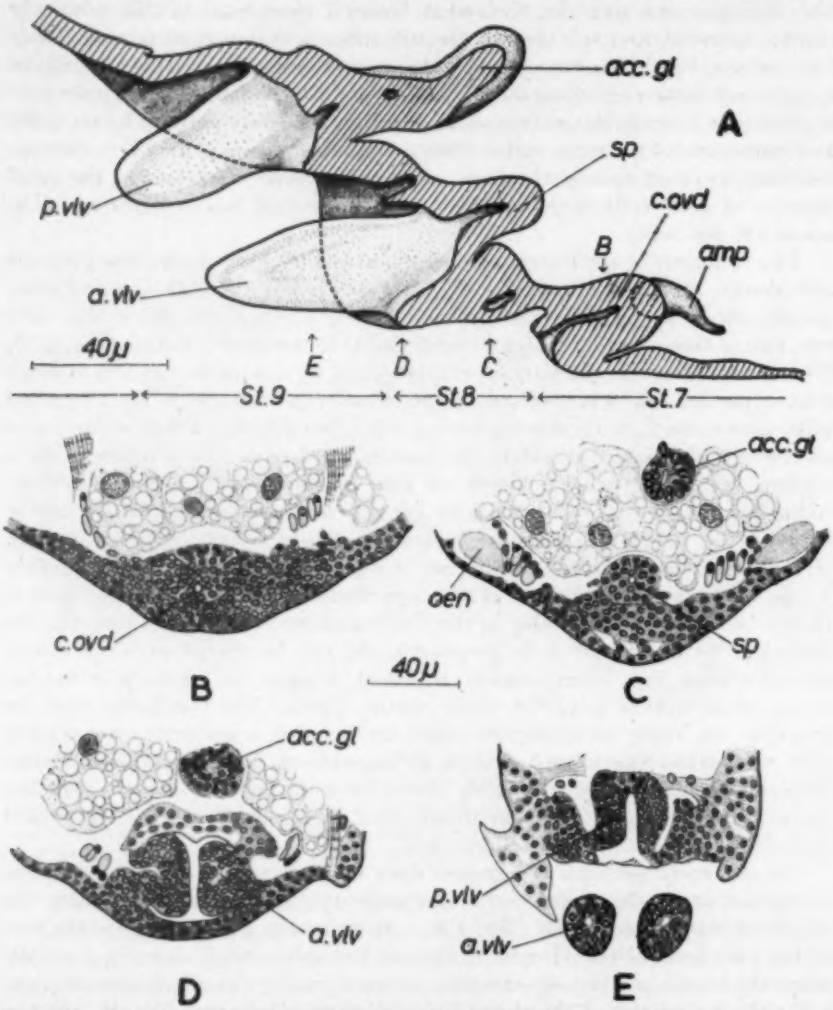

Fig. 4—Female genital region of young prepupa. A. Reconstruction in sagittal plane, approximate segmental boundaries indicated. B, C, D, & E. Transverse sections at points indicated by arrows. B. Through common oviduct rudiment. C. Through spermathecal rudiment. D. Through bases of anterior ovipositor valves. E. Through bases of posterior ovipositor valve rudiments at opening of accessory gland rudiment.

acc. gl, accessory gland rudiment ; *amp*, genital ampulla ; *a.vlv*, anterior ovipositor valve ; *c.ovd*, common oviduct rudiment ; *oen*, oenocyte ; *p.vlv*, posterior ovipositor valve ; *sp*, spermathecal rudiment.

walls of the cavity are much thickened and form the bases of the anterior valves which project backwards beyond the eighth segment. From the front of the cavity the spermathecal rudiment projects forwards, its small lumen continuous with the larger space between the anterior valve rudiments. The dorsal wall of the cavity is continuous with the ninth sternum which is infolded medially (Fig. 4 E). At the front of this infolding a conspicuous and virtually solid ingrowth of epidermal cells forms the early rudiment of the accessory gland (Figs. 4 A, C & D), while the lateral walls of the infolding make up the bases of the posterior ovipositor valves. By the pharate pupa (Figs. 5 & 6) all four valves have grown in length and their distal ends protrude out of the almost pocket-like cavities from whose inner lateral walls their basal regions arise.

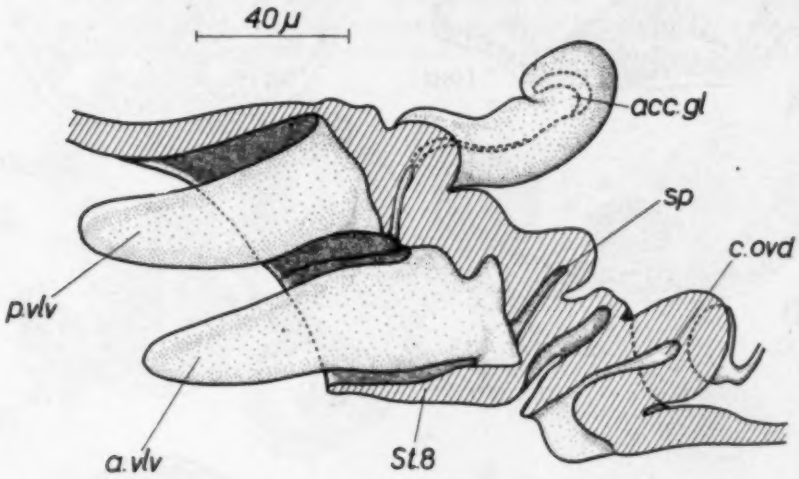

Fig. 5—Reconstruction of genital area of pharate pupa.

acc.gl, accessory gland rudiment ; *a.vlv*, anterior ovipositor valve ; *c.ovd*, common oviduct rudiment ; *p.vlv*, posterior ovipositor valve ; *sp*, spermathecal rudiment ; *St.* 8, eighth abdominal sternum.

Throughout this period the epidermis of the valve rudiments is several layers thick ; they are oval or rounded in cross-section, solid or with only a small lumen, and remain free from each other for their whole length (Figs. 4 E, 6 D–F). The small spermathecal rudiment changes little in shape or position (Figs. 4 C, 6 C) but the accessory gland rudiment grows in length, its anterior end becomes recurved and indications of a lumen develop in it. This lumen opens posteriorly on a small, more or less conical protruberance just in front of the bases of the posterior valves (Fig. 6 E). In the anterior recurved part of the rudiment the lumen expands somewhat and the early differentiation into the duct and vesicle of the accessory gland is thus indicated. Histologically, however, all the rudiments of the efferent duct system and its annexes still consist of undifferentiated cells resembling those of which the larval discs were composed.

During the prepupa early stages in the development of certain ovipositor muscles also become apparent. Irregular masses of myoblasts—strongly basiphil nuclei embedded in a less basiphil cytoplasm—are shown in Fig. 6 E (*m*), while a rather more advanced stage of myogenesis (in a different muscle) is shown in Fig. 4 E, where the transverse alignment of developing fibres is in progress on each side of the posterior valve rudiments. A fuller account of the development of the ovipositor muscles is given below (p. 427).

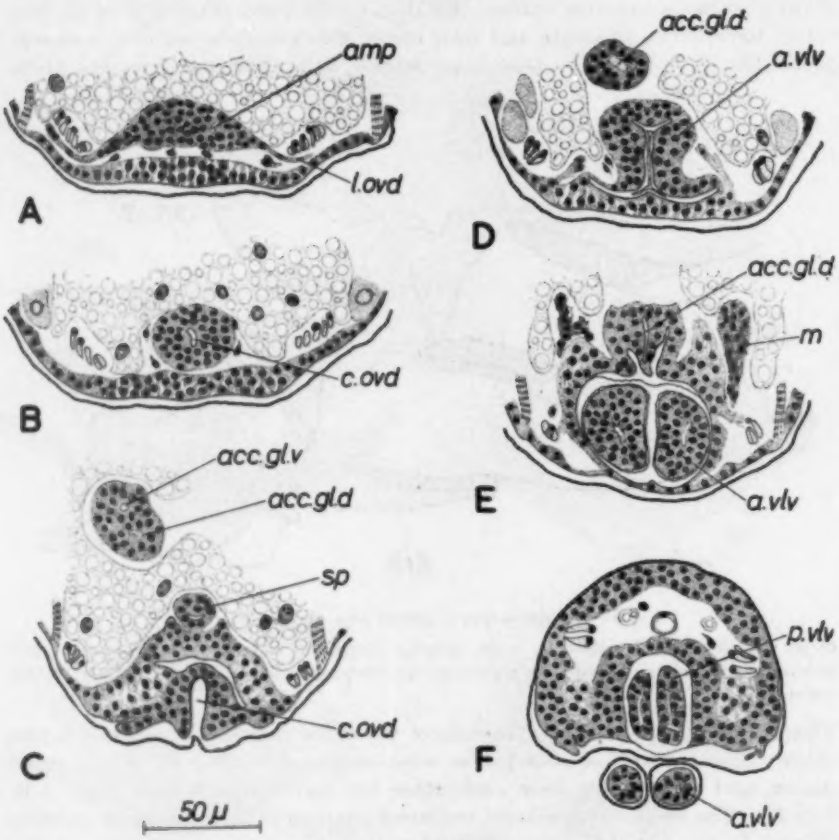

Fig. 6—Transverse sections of genital region of pharate pupa. A. Through genital ampullae and seventh abdominal sternum. B. Through common oviduct rudiment and seventh sternum. C. Through spermathecal rudiment, eighth sternum, and seventh sternum with opening of common oviduct rudiment. D. Through eighth sternum and base of anterior ovipositor valves. E. Through opening of accessory gland duct and near origin of posterior ovipositor valves, anterior valves free. F. Through posterior part of ninth abdominal sternum, anterior and posterior valves free.

acc.gl.d, duct of accessory gland ; *acc.gl.v*, vesicle of accessory gland ; *amp*, genital ampulla ; *a.vlv*, anterior ovipositor valve ; *c.ovd*, common oviduct ; *l.ovd*, lateral oviduct ; *m*, developing muscle ; *p.vlv*, posterior ovipositor valve ; *sp*, spermathecal rudiment.

PUPA AND PHARATE ADULT

The principal changes occurring during the pupa are the complete subdivision of the ovaries and the formation of the vagina, accompanied by further differentiation of the accessory gland. During the pharate adult there is progressive differentiation of all structures towards the imaginal condition, though the ovarioles remain immature and the lateral oviducts only acquire a lumen at the very end of this period.

During the earlier part of the pupal stadium the subdivision of the ovary into four ovarioles is completed for all except the extreme posterior region (Fig. 7). Division proceeds from before backwards and is accompanied by the serial arrangement one behind the other of the two or three largest (i.e. most posterior) oocytes. Subsequently the follicular tissue, in which mitoses are still occurring, forms a uniform single-layered sheath around the older oocytes, interfollicular cells begin to move into position, and by the early pharate adult

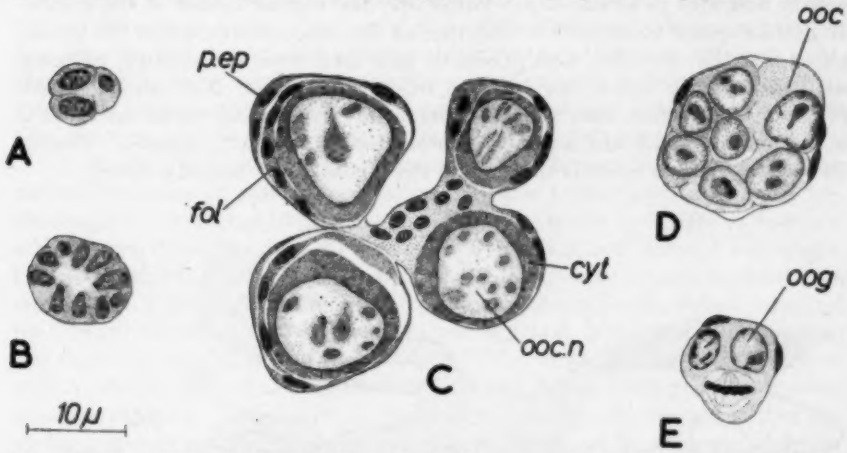

Fig. 7—Ovary and lateral oviduct of old pupa, all in transverse section. A. Through main part of lateral oviduct rudiment. B. Through lateral oviduct near junction with ovary. C. Posterior part of ovary through basal oocyte of each of four ovarioles. D. An ovariole, anterior region. E. An ovariole, near apex, showing oogonial mitosis.
cyt, cytoplasm of primary oocyte ; *fol*, follicular cells ; *ooc*, primary oocyte ; *ooc.n*, nucleus of oocyte ; *oog*, oogonium ; *p.ep*, primary epithelial sheath.

the division into ovarioles is complete. The larger oocytes do not, however, increase in size during this period and even in the oldest pharate adult examined they still average only about 15 μ broad and 20 μ long. These older oocytes occupy the posterior two-fifths or so of each ovariole while the somewhat broader anterior part (about 20 μ in diameter) contains irregularly arranged oogonia or very young oocytes with prefollicular or incipient follicular tissue. The total number of germ-cells continues to increase slightly during the pupal period but the ovarioles proper do not change much in length ; excluding the terminal filaments they are about 100 μ long in the pharate adult. The terminal filaments, however, grow anteriorly ; by about the middle of the pupal stage the common filament has reached the second abdominal segment, and by the

pharate adult there is a sharper distinction between the terminal filament and the anterior part of each ovariole. The very delicate filament of each ovariole then consists mostly of a single chain of cells and the eight filaments continue separately into the second abdominal segment, running pressed between the dorsal wall of the gut and the immediately overlying lobe of fat-body. Anteriorly all eight filaments unite but apparently do not extend into the thorax.

The slight posterior extension of the ovary and the more pronounced forward growth of the common oviduct rudiment together result in a considerable shortening of the lateral oviduct rudiments during the pupa. Whereas in the prepupa and pharate pupa these are about 150 μ long, by the pharate adult they have decreased to about 70 μ or less. At the same time the lateral oviducts increase slowly in diameter from about 3–6 μ to about 6–9 μ and near the ovary they have expanded in the pharate adult to a small calyx-like region with a diameter of about 15 μ. Posteriorly the narrow lumen of the genital ampulla acquires a connection with that of the common oviduct in the young pupa, and the ampullae then gradually lose their separate identity, merging with the anterior end of the common oviduct rudiment. The posterior part of the lateral oviduct later increases still further in diameter (to about 10–12 μ in the pharate adult) and a narrow lumen develops (about 3 μ across). Finally the delicate lumen extends throughout the whole of the lateral oviduct.

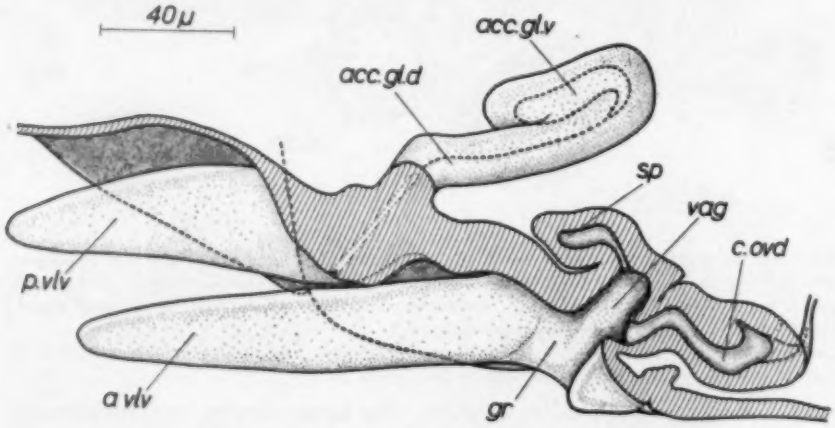

Fig. 8—Reconstruction of genital region of young pupa.
acc.gl.d, duct of accessory gland ; *acc.gl.v*, vesicle of accessory gland ; *a.vlv*, anterior ovipositor valve ; *c.ovd*, common oviduct ; *gr*, groove of eighth sternum forming vagina ; *p.vlv*, posterior ovipositor valve ; *sp*, spermatheca ; *vag*, vagina.

During the pupa the changes which occur in the ectodermal efferent duct rudiments of the seventh and eighth abdominal segments are so closely interrelated that they must be considered together. By the early pupa (Figs. 8 & 10), the common oviduct rudiment has increased in length and its walls have become somewhat thinner, though still two or more cell-layers thick. Its lumen is

now larger and transversely elongated in cross-section, and is connected with the lumina of the ampullae (Fig. 9 B). Posteriorly the opening of the common oviduct has been carried backwards by the ventral closure of the lateral lips of the groove found on the seventh sternum of the prepupa, and it now runs into a chamber which represents the beginnings of a vagina (Figs. 9 C & D). This broadly open vaginal cavity is a derivative of the eighth sternum whose formation requires some explanation. At about the time of the moult from prepupa to pupa the epidermis of the median part of the eighth sternum becomes retracted forwards and dorsalwards, while the more lateral epidermis of the sternum forms the side walls of a deep broad groove (Fig. 9 D, *gr*). Anteriorly this groove has already begun to close over by ventral fusion of its lips ; posteriorly it is still widely open between the bases of the anterior ovipositor valves (Figs. 9 C–E). The spermathecal rudiment, whose lumen has now expanded distally, connects with the anterior part of the vaginal cavity by a short duct, the opening of which has been carried forwards by the medial retraction of the epidermis of the eighth sternum. The early pupa, in fact, sees the end of the eighth abdominal sternum as a structure in its own right. Much of it is being converted into the vagina, while the lateral parts of its epidermis remain external and will form what Scudder (1960) regards as the first gonocoxae of the adult (the ' eighth sternum ' of Jones (1954)).

From this situation in the young pupa it is easy to trace the further development of the common oviduct and vagina. In later pupal stages (Figs. 10 & 11), the common oviduct continues to expand transversely and its wall becomes still thinner, especially on the ventral side where it is now reduced to a single-layered epithelium (Figs. 11 A & B). At the same time ventral fusion of the lateral lips of the vaginal cavity convert the latter into a depressed tube and its forward extension well into the seventh segment brings it to lie dorsal to the posterior part of the common oviduct (Fig. 10). The result of this is a shift in the junction of the common oviduct and vagina leading to the appearance shown in Fig. 11 C. Here, in the area of junction, the ventral wall of the median efferent duct consists of the thin-walled common oviduct, while its dorsal region is made up of the thicker vaginal wall. The same process also results in the anteriormost part of the vagina, into which the spermathecal duct opens, forming a kind of vestigial bursa copulatrix (see Fig. 10 and the dorsal unlabelled cavity in Fig. 11 B). Meanwhile the lumen of the spermatheca becomes more clearly differentiated into a duct-like part and a globular distal region, and its wall becomes attenuated to a single layer of cells (Fig. 11 C).

The development of the accessory gland during the pupa is quite simple (Figs. 8 & 10). In the young pupa there is already a clear distinction into a small vesicle with a conspicuous central lumen, and a relatively short duct in which the lumen is poorly developed but opens at the apex of a large protuberance between the bases of the posterior ovipositor valves. The duct approximately doubles in length during the stadium and the vesicle enlarges somewhat. The cells of which the rudiment is composed are still undergoing mitosis in the young pupa (Fig. 9 D), but this ceases later and the walls of the gland become reduced to a single layer (Figs. 11 A & F). Posteriorly, the prominence bearing the opening of the duct becomes drawn out into a back-

wardly directed conical papilla ; this is fused basally with the conjoint basal region of the posterior ovipositor valves but its apex projects freely between the valves (Figs. 11 G & H).

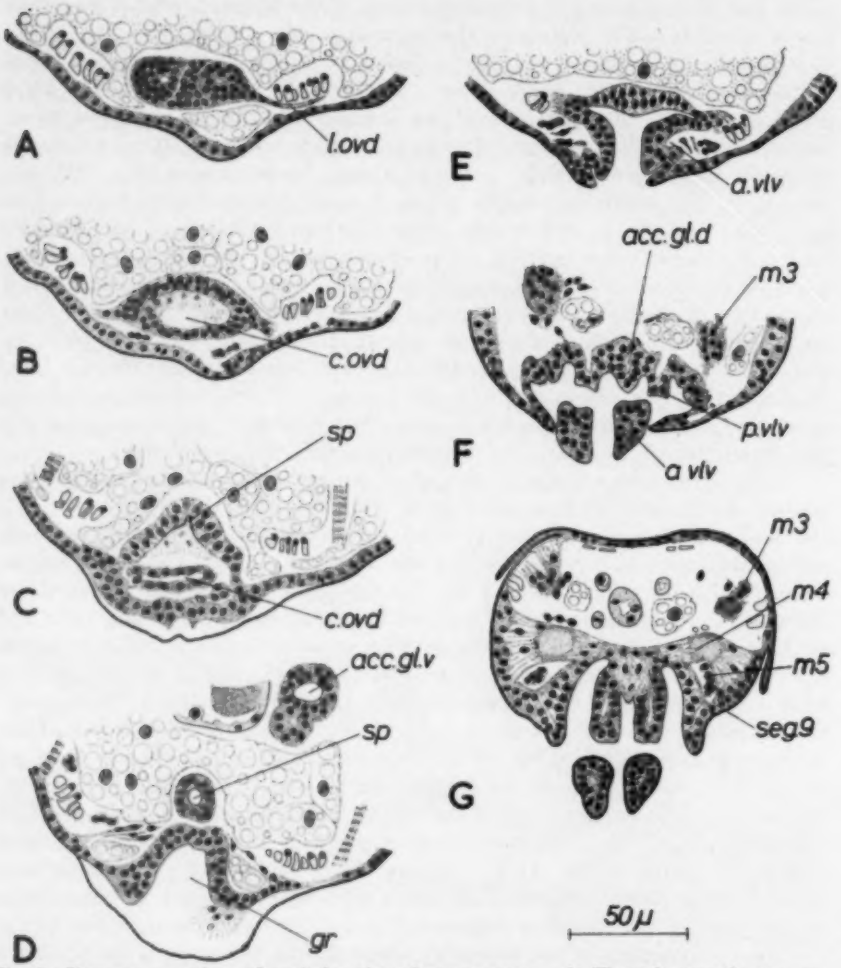

Fig. 9—Transverse sections of genital region of young pupa. A. Through seventh sternum, genital ampullae and anterior end of common oviduct rudiment. B. Through common oviduct rudiment. C. Through vagina near junction with common oviduct and at entrance to spermatheca. D. Vaginal groove (eighth sternum) and spermatheca, with accessory gland rudiment. E. Through eighth sternum at base of anterior ovipositor valves. F. Through ninth sternum at base of posterior ovipositor valves and exit of accessory gland duct. G. Near junction of eighth and ninth terga, showing ovipositor valves and myogenesis.

acc.gl.d, duct of accessory gland ; *acc.gl.v*, vesicle of accessory gland ; *a.vlv*, anterior ovipositor valve ; *c.ovd*, common oviduct ; *gr*, groove on eighth sternum forming vagina ; *l.ovd*, lateral oviduct ; m_3-m_5, rudiments of developing adult muscles ; *p.vlv*, posterior ovipositor valve ; *seg.* 9, ninth segment ; *sp*, spermatheca.

The moult into the pupa allows the lengthening ovipositor valves to be fully everted from the pockets in which their basal third had previously been contained. At the same time the posterior part of the ninth sternum and most of the tenth sternum become more extensively infolded to accommodate the ovipositor (Figs. 9 G, 11 H & I). The valves themselves do not change greatly in histological structure during the pupa, though their lumina become rather larger. The formation of the vagina, however, and the associated general forward shift of the ventral genital region, lead to some changes in the relations of the valves. Thus, the bases of the anterior ones become rather more widely spaced and assume a close relationship with the inner surface of the first gonocoxal region as this becomes differentiated (Figs. 9 E, 11 E). Meanwhile the extension of the posterior valves occurs in such a way that their inner dorsal surfaces are fused with each other, and also with the papilla of the accessory gland, for about the basal third of their length.

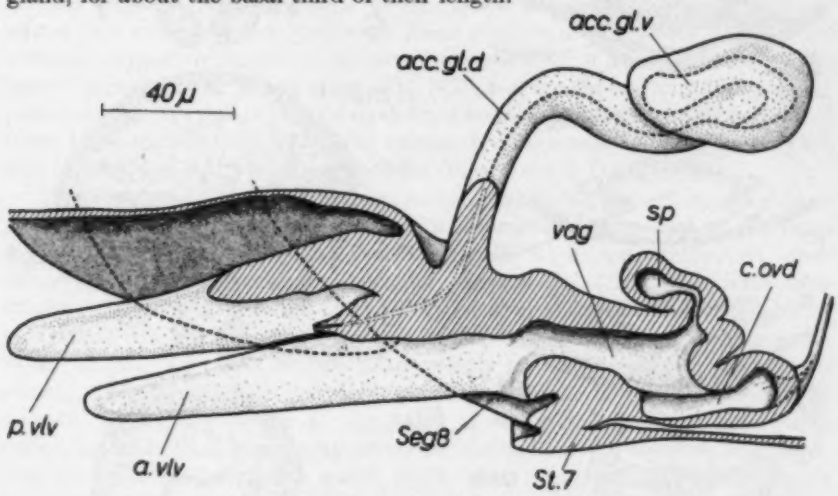

Fig. 10—Reconstruction of genital region of old pupa.

acc.gl.d, duct of accessory gland ; *acc.gl.v*, vesicle of accessory gland ; *a.vlv*, anterior ovipositor valve ; *c.ovd*, common oviduct ; *p.vlv*, posterior ovipositor valve ; *Seg.8*, lateroventral part of eighth segment (first gonocoxa of Scudder) ; *sp*, spermatheca ; *St.7*, seventh abdominal sternum ; *vag*, vagina.

Certain changes in the musculature of the genital segments must now be discussed. The ventral longitudinal muscles of the seventh abdominal segment of the larva are retained in a normal histological condition throughout the life-cycle, but their position changes considerably from the later pupa onwards. Their posterior attachments to the hypertrophied epidermis from which the common oviduct arises become carried forwards as the latter extends anteriorly. The attachments lie near the junction of the common oviduct with the overlying vagina and as this too grows forwards the muscle gradually begins to assume a diagonal position, running posteromedially from the anterolateral boundary of the seventh sternum to the junction. This rotation of the muscle

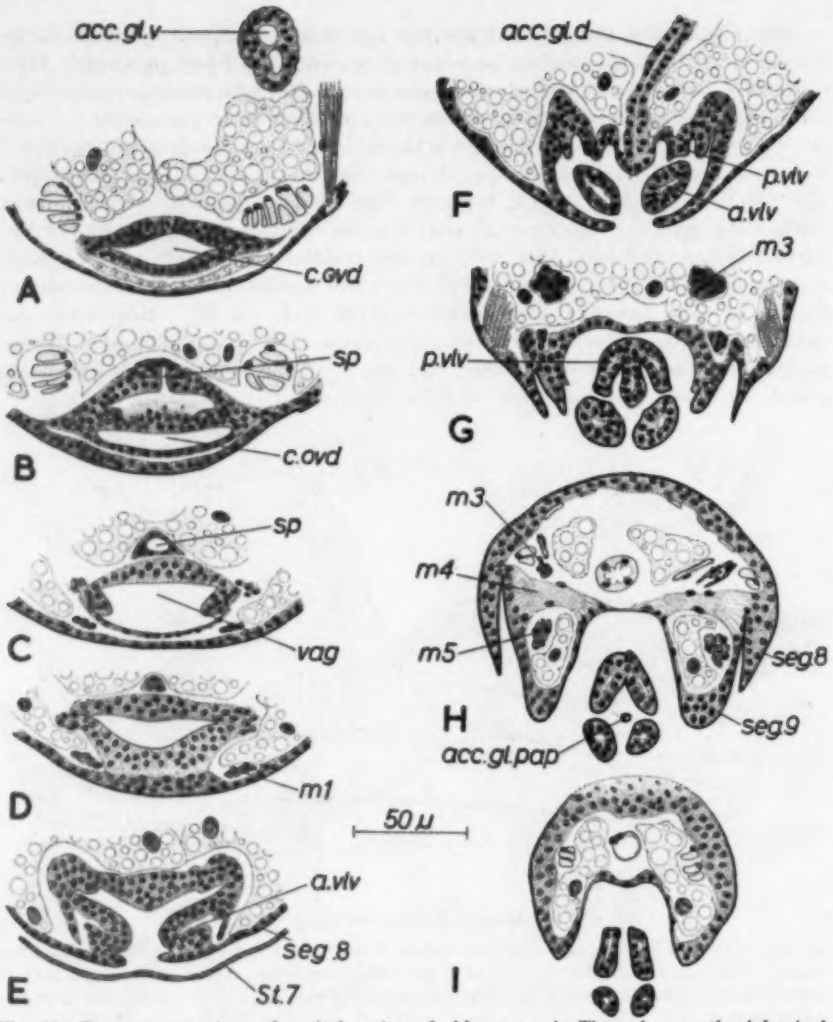

Fig. 11—Transverse sections of genital region of old pupa. A. Through seventh abdominal sternum near junction of common oviduct and ampullae, accessory gland dorsolaterally. B. Posterior to A, showing 'bursa' and opening of spermathecal duct, both lying dorsal to common oviduct. C. At junction of common oviduct and vagina, showing spermatheca. D. Through vagina posterior to spermatheca. E. Through bases of anterior ovipositor valves. F. Through base of posterior ovipositor valves and opening of accessory gland duct. G. Posterior to F, near lateroventral junction of eighth and ninth segments. H. Posterior to G, showing free anterior valves, fused posterior valves, papilla of accessory gland, and developing ovipositor muscles. I. Posterior to H, through back of ninth segment.

acc. gl.d, duct of accessory gland ; *acc.gl.pap*, papilla of accessory gland ; *acc.gl.v*, vesicle of accessory gland ; *a.vlv*, anterior ovipositor valve ; *c.ovd*, common oviduct ; m_1, m_{2-3}, developing ovipositor muscles (see text for explanation) ; *p.vlv*, poterior ovipositor valve ; *seg.* 8, *seg.* 9, eighth and ninth abdominal segments ; *sp*, spermatheca ; *St.*7, seventh abdominal sternum ; *vag*, vagina.

has the important result that for the first time the lateral oviducts are no longer pinned beneath its posterior part, and are therefore free to expand into wide distensible channels for the eggs in the mature adult. Furthermore, the changing position of the muscle results in a change of function. It is no longer a retractor of the seventh sternum but will become an extrinsic oviducovaginal dilator, and it is supplemented by other fibres which have already begun to develop directly from new myoblasts running between the lateroventral epidermis at the back of the seventh segment and the walls of the vaginal groove. Fig. 9 D shows these myoblasts and Figs. 11 A–D illustrate how the original ventral longitudinal muscles of the seventh segment no longer run to the back of the sternum ; they are actually attached to the junction of oviduct and vagina immediately anterior to the section depicted in Fig. 11 C. The adult, however, also possesses normal ventral longitudinal muscles of the seventh segment (cf. Figs. 14 A & B). These are newly-formed structures whose first rudiments are visible as small clusters of myoblasts beneath the common oviduct rudiment of the pharate pupa (Fig. 6 A). They are a little better defined in the young pupa (Fig. 9 B) and by the old pupa they have, posteriorly at any rate, formed well-defined but still small and strongly basiphil fibres lying immediately above the epidermis of the seventh sternum on each side of the vagina and common oviduct (Figs. 11 C & D, m_1).

The changes in the musculature of the eighth abdominal segment have proved less easy to follow, but the ventral longitudinal muscles of the larva persist up to the early pupa—where they are becoming attenuated—then histolyse and are absent from the older pupal stages. The other ovipositor muscles of this segment in the adult are an anterior and a more lateral posterior pair which run from near the junction of the first gonocoxa and gonangulum to the tergum. The first of these is apparently formed from an intersegmental muscle of the larva which originally ran from near the boundary between the seventh and eighth sterna to the front of the eighth tergum, and which maintains its normal histological structure throughout the prepupa and pupa. The other is a similar muscle which, in the larva, ran from the eighth tergum to sternum and whose ventral attachment has been carried forwards in the formation of the vagina and first gonocoxa. Certainly there is no evidence of new muscles arising within the eighth segment during the pupal stage. In the ninth segment, on the other hand, the main event is the formation *de novo* of three conspicuous paired muscles. Two of these are already indicated as groups of myoblasts in the prepupa and now continue their growth and differentiation. There is firstly the transverse muscle from the back of the infolded ninth sternum to the sides of the segment where the epidermis is thickening to form the gonangulum ; see Figs. 9 G & 11 H, m_4. The second developing muscle runs from the base of the gonangulum rudiment near the origin of the posterior valve backwards and dorsalwards to the front of the ninth tergum (Figs. 9 F & G, 11 G and H, m_3). For much of its course this runs close to the persistent ventral longitudinal muscle of the ninth segment. A third muscle rudiment is first visible in the early pupa and runs from the lateroventral part of the ninth tergum below the gonangular thickening up to the dorsal wall of the tergum (Figs. 9 G, 11 H, m_5).

No changes of morphological importance occur in the pharate adult, as can be seen by comparing the reconstruction of the late pupa (Fig. 10) with that of the pharate adult (Fig. 12, in which the pupal cuticle enclosing the whole structure is not illustrated). The wall of the common oviduct and vagina becomes thinner, and the transverse flattening of its lumen becomes even more pronounced ; the spermatheca and its lumen grow somewhat ; and the

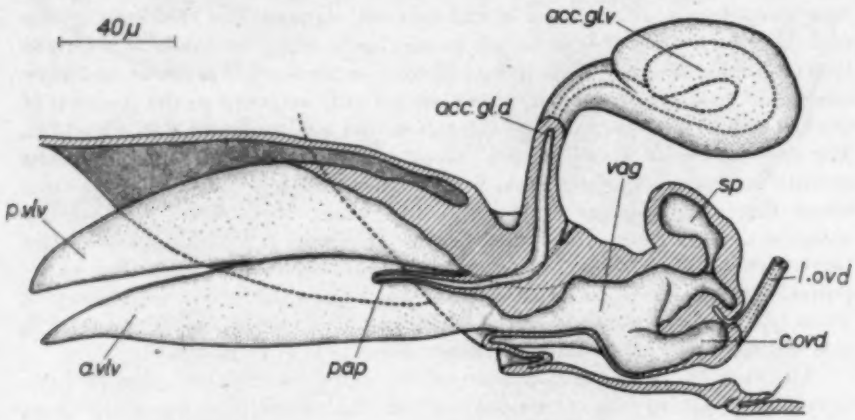

Fig. 12—Reconstruction of genital region of pharate adult.

acc.gl.d, duct of accessory gland ; *acc.gl.v*, vesicle of accessory gland ; *a.vlv*, anterior ovipositor valve ; *c.ovd*, common oviduct ; *l.ovd*, lateral oviduct ; *pap*, papilla of accessory gland ; *p.vlv*, posterior ovipositor valve ; *sp*, spermatheca ; *vag*, vagina.

accessory gland increases appreciably in size. The lumen of the vesicle and duct also enlarge but they remain devoid of secretion and the cells lining them do not yet show any signs of secretory activity. The papilla of the accessory gland becomes markedly attenuated and a slight dilation of the duct occurs at its base. The internal cavities of the ovipositor valves also enlarge as their epidermis begins to assume a single-layered structure and from being elongate-oval in transverse section they begin to take on the more angulate appearance of the adult. The adult cuticle is now being laid down and is most conspicuous over the accessory gland papilla, the posterior part of the accessory gland duct and the ovipositor valves. At their bases, where the anterior and posterior valves of the adult are in contact, the ventral ridge of each posterior valve is fitted into the dorsal groove of the corresponding anterior one. More distally, these ridges and grooves are forming but are prevented from engaging by the intervening layers of pupal cuticle.

MATURE ADULT

From Figs. 13 and 14 it may be seen that appreciable post-metamorphic changes occur before the adult is finally mature. When it first emerges the adult has ovaries which do not differ greatly in size and structure from those seen in the older pupal stages (cf. also Lewis, 1959). Subsequent growth of the

oocytes through yolk-deposition is accompanied by distension of the lateral oviducts, in which mature eggs may be found after ovulation (Fig. 13). The oviduct and vagina are also now greatly enlarged with a very thin epithelial layer and a delicate cuticular intima (Figs. 14 A and B); the oviduco-vaginal dilator muscles have attained their final, almost transverse, orientation; and the thin-walled spermatheca of all mature females sectioned contains a mass of spermatozoa. It may perhaps be mentioned here that a claim by Pussard-Radulesco (1931) that females of *Limothrips denticornis* copulate only in the prepupal and pupal instars is not supported by this work on *L. cerealium*,

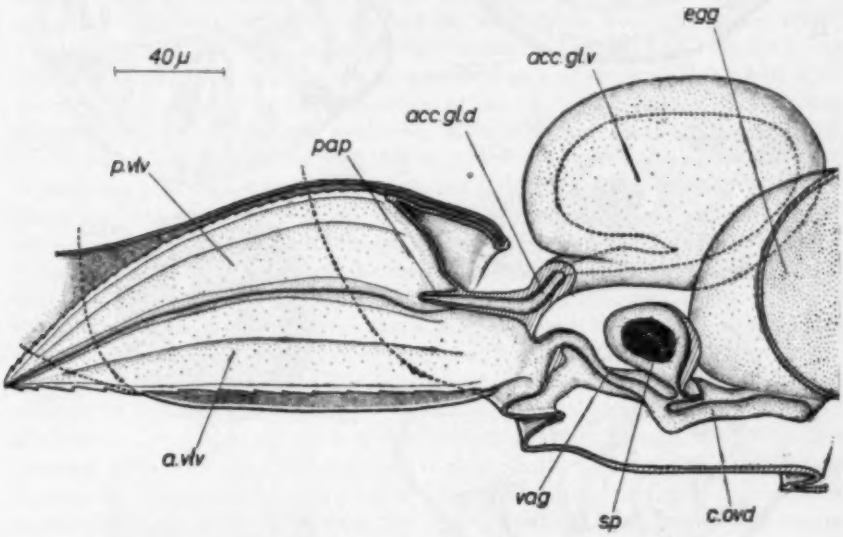

Fig. 13—Reconstruction of genital region of adult.

acc.gl.d, duct of accessory gland; *acc.gl.v*, vesicle of accessory gland; *a.vlv*, anterior ovipositor valve; *c.ovd*, common oviduct; *egg*, mature egg in egg-calyx (lateral oviduct); *pap*, papilla of accessory gland; *p.vlv*, posterior ovipositor valve; *sp*, spermatheca; *vag*, vagina.

where the spermatheca of these immature stages was always empty. The vesicle of the accessory gland becomes considerably dilated after emergence and is then filled with a finely granular eosinophil secretion. In the more distended parts of the vesicle of the mature adult the epithelium is approximately cubical and strongly basiphil, though without secretion-containing vacuoles. Towards the duct, however, the cells are taller and contain one or more large distal globules of secretion (Fig. 14 F). Similar cells also occur in the anterior section of the duct, which must therefore be regarded as partly secretory in function (Fig. 14 E). Posteriorly, however, the epithelium of the duct is much thinner, the cells are less closely-spaced, and the intima is strongly thickened with helical ridges (Fig. 14 C). Immediately before entering the papilla the duct is somewhat dilated and the intima here is uniform, even thicker, and lightly pigmented. Further thickening and pigmentation of the

cuticle in the young adult give rise to the rigid ovipositor and its basal articulatory sclerites (Fig. 14 C & D), while the newly-formed ovipositor muscles described above assume the same fibrous eosinophil appearance characteristic of those other, larval, muscles which had retained their histological integrity throughout the prepupal and pupal instars.

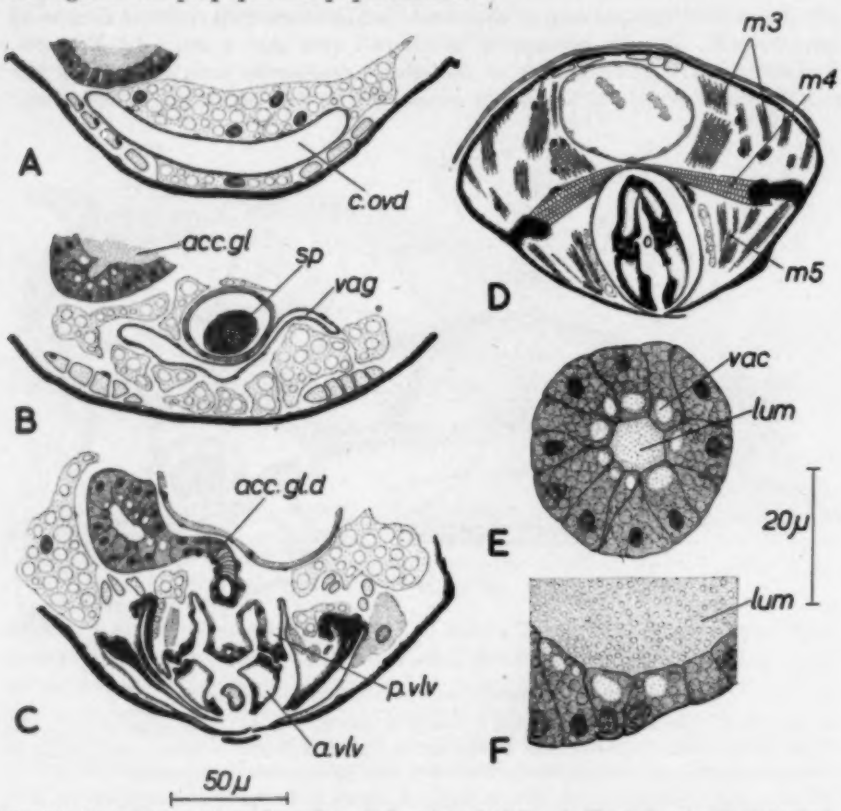

Fig. 14—Transverse sections through genital region of adult. A. Through seventh abdominal segment, showing common oviduct ; B. Through seventh abdominal segment, showing vagina and spermatheca. C. Through eighth abdominal segment showing accessory gland duct, anterior valves and base of posterior valves of ovipositor. D. Through junction of eighth and ninth segments, showing ovipositor. E. Through anterior part of accessory gland duct, showing secretory cells. F. Secretory cells in wall of accessory gland vesicle.
acc.gl, accessory gland ; *acc.gl.d*, accessory gland duct ; *a.vlv*, anterior ovipositor valve ; *c.ovd*, common oviduct ; *lum*, lumen of accessory gland ; $m_{3\text{-}5}$, ovipositor muscles (see text for explanation) ; *p.vlv*, posterior ovipositor valve ; *sp*, spermatheca ; *vac*, vacuole of accessory gland epithelium ; *vag*, vagina.

DISCUSSION

When Heberdey (1931) came to review the morphology of the female reproductive system of the terebrantian Thysanoptera and to compare it with that of other insects, he was forced to rely on the meagre ontogenetic data of

Jordan (1888) and on the comparative anatomy of the adult. It is now possible to see from this account of *Limothrips cerealium* that the main features of development are very similar to those reported in many other exopterygote insects, especially among the relatively well investigated orthopteroid groups. Thus, the formation of the common oviduct from a median ingrowth at the back of the seventh abdominal segment also occurs, according to Nel (1930) and Qadri (1940), in *Nemoura* and other Plecoptera, in *Blatta* and *Blattella*, in various Orthoptera *sensu stricto* like *Locustana*, *Colemania*, *Locusta*, *Acheta* and *Leptophyes*, and in *Forficula*, while George (1928) reports the same process in the auchenorrhynchan homopteran *Philaenus*. This appears to be the primitive condition in insects and where, as in some endopterygotes which will not be discussed here in detail, the ' common oviduct ' apparently arises from the eighth sternum it must be regarded as a specialized feature of their development. Similarly, the formation of the vagina by closure of a median groove on the eighth sternum occurs in *Chloroperla* and *Locusta* (Qadri, 1940), in *Colemania* and *Locustana* (Nel, 1930), and in *Philaenus* (George, 1928), and is probably best regarded as a common derivative of the condition in which the primitive gonopore or gonopores remain open behind the seventh abdominal sternum and no true vagina is formed, as in the Ephemeroptera and Dictyoptera. Turning now to the origin of the spermatheca, one finds that this organ is probably not always homologous in different insects (Berlese, 1909). Though the spermatheca develops from the eighth segment in most of the orthopteroids cited above, an origin from the seventh sternum is found in the mayfly *Heptagenia* (Qadri, 1940) and allegedly also in *Philaenus* (George, 1928). And whereas in *Blatta* it arises from the eighth segment, the atypical spermathecae of *Blattella* develop at the back of the seventh sternum, though an evanescent ingrowth from the eighth also occurs in development (Nel, 1930). Evidently *Limothrips* resembles the majority of investigated exopterygotes in having a spermatheca which develops from the eighth abdominal segment. The origin of the accessory gland from an ingrowth on the ninth sternum also represents a widespread condition in the Exopterygota : only in species where the adult has no such gland (not even in a vestigial state) is an invagination lacking there.

In those species of insects with a complete ovipositor it is well known that the anterior valves (first gonapophyses of Scudder, 1957) arise from the eighth sternum while the two posterior pairs (second gonapophyses and gonoplacs) are formed on the ninth segment. The main question in the terebrantian Thysanoptera concerns the identity of the single posterior pair of valves. These have been regarded, without much evidence, as the second gonapophyses (Heberdey, 1931 ; Jones, 1954). Dr G. G. E. Scudder, who has kindly allowed me to see the manuscript of his unpublished paper on the comparative morphology of the insect ovipositor, also adopts this interpretation, which is now based on his identification of the basal sclerites of the ovipositor and their associated muscles. The development of the ovipositor of *Limothrips* does not, in itself, provide any new confirmatory evidence. There is, however, in the pupa, a pair of distinct lateral protruberances between the bases of the posterior valves and the adjacent overhanging lateroventral part of the body-wall of the ninth segment. These structures, of sternal origin, might conceivably be

regarded as the transient rudiments of the gonoplacs, but I hesitate to claim this in the absence of supporting data. As to the formation of the basal articulatory sclerites of the ovipositor, these only begin to attain their definitive adult form in the preceding pharate phase : prior to that the epidermis is so thickened and so devoid of clearly-defined boundaries that it is difficult to delimit their precise sites of origin. It is, however, evident that the conspicuous first gonocoxae of the adult are the lateral remnants of the eighth sternum, left after the formation and anterior displacement of the vagina ; and it is also interesting to note that the gonangulum appears to be foreshadowed during the prepupa and pupa by a proliferated strip of epidermis whose surrounding regions form the ninth tergum of the adult.

A final, and unsolved, morphological problem concerns the precise boundary between the mesodermal and ectodermal parts of the efferent duct system. It has already been pointed out (p. 419) that the origin of the genital ampullae of *Limothrips* is uncertain, and the same is true of practically all other investigated insects, in which the mesodermal nature of these structures seems to be assumed rather than demonstrated. Even where, as in some orthopteroid insects (e.g. Nel, 1930 ; Qadri, 1940), the ampullae are present before the common oviduct rudiment arises, this does not establish their mesodermal character, since Heberdey (1931) states that in some Coleoptera they arise from the epidermis earlier than and independent of the common oviduct. And Abul-Nasr (1950) claims to have demonstrated an encroachment of ectodermal on to mesodermal tissues in this region of some nematoceran Diptera, with the ampullae as epidermal derivatives. In any case, with the incorporation of the ampullae into the anterior part of the common oviduct during the pupa of *Limothrips*, it becomes even more difficult to find a landmark between ostensibly mesodermal and ectodermal rudiments, as there is no histological distinction between the two kinds of tissue. The whole subject seems to require further study, especially in the more generalized insects where the ampullae are formed in the embryo.

Although the above morphological interpretations show that the terebrantian Thysanoptera are basically similar to many of the less specialized Exopterygota, the time-relations of the developmental processes are very different in the two groups. Thus, to take only a few examples, the division of the ovary rudiment into ovarioles begins only at about the moult into the pupa in *Limothrips*, whereas clear indications of subdivision are present in the first instar of forms like *Blattella* (Heymons, 1891), *Bacillus* (Daiber, 1904), *Locusta* (Roonwal, 1937) or *Oncopeltus* (Wick & Bonhag, 1955), and in the second instar of *Melanoplus* (Nelsen, 1934) and *Liposcelis* (Goss, 1954). Similarly, whereas the ectodermal parts of the efferent duct system all arise during the second half of post-embryonic life in *Limothrips*, their beginnings may be seen as early as the first instar of *Locusta*, *Locustana*, and *Colemania* or the second instar of *Forficula* and *Blatta* (Nel, 1930 ; Qadri, 1940). Again, the closure of the vaginal groove, occurring in the pupa of *Limothrips*, takes place in the first instar of *Locustana* and *Colemania*, while the establishment of a lumen in the lateral oviducts of *Limothrips* is yet another process in which there is much delay. Though it is well known that the various parts of the reproductive system arise at different

times and grow at different rates in different insects, the conclusion is clear that there is a general tendency in *Limothrips* for these organs to arise relatively late and thereafter to develop relatively quickly. In this respect it obviously resembles the Endopterygota. In fact, by comparing the important morphogenetic events in the prepupa and pupa of *Limothrips* with those described in the pupae of various Endopterygota by Metcalfe (1932), Murray & Tiegs (1935), D'Rozario (1942), Dean (1943), Abul-Nasr (1950), Brunold (1957) and others, it becomes clear that the prepupa and pupa of the Thysanoptera together correspond closely to the Endopterygote pupa. This is not, perhaps, a very surprising conclusion, but to have demonstrated it in detail for one system may be regarded as an advance in our understanding of the relations between the unusual life-cycle of the Thysanoptera and those of more typical hemimetabolous and holometabolous insects.

SUMMARY

1. The development of the female reproductive system of *Limothrips cerealium* Haliday was studied between the second-instar larva and the mature adult.

2. The undifferentiated ovary rudiments of the larva divide into ovarioles by the early pupa but further growth of the oocytes is largely post-metamorphic.

3. The lateral oviduct rudiments remain a solid strand of cells until the pharate adult ; genital ampullae have developed by the early prepupa and merge with the common oviduct rudiment in the late pupa.

4. The common oviduct has arisen from a median invagination at the back of the seventh abdominal sternum by the early prepupa. In the pupa it becomes associated posteriorly with the vagina which forms through ventral closure of a groove on the eighth sternum. The spermatheca is derived from a median ingrowth on the eighth sternum, the accessory gland from one on the ninth.

5. The ovipositor valves originate in the older second-instar larvae, the anterior pair from the eighth sternum and the posterior pair from the ninth. Some ovipositor muscles arise through aggregation of myoblasts in the prepupa or pupa, some through changes in the position of otherwise unaltered larval muscles.

6. The morphology of the reproductive system is essentially similar to that of many less specialized Exopterygota, but its delayed development recalls Endopterygote morphogenesis. The events which occur in the prepupa and pupa of *Limothrips* are broadly equivalent to those taking place in the pupa of Endopterygote insects.

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OBSERVATIONS ON THE ECOLOGY AND LIFE HISTORY OF THE FAIR ISLE FIELD-MOUSE *APODEMUS SYLVATICUS FRIDARIENSIS* (KINNEAR)

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(With 5 figures in the text)

A total of 120 field-mice from Fair Isle (*Apodemus sylvaticus fridariensis* (Kinn.)) have been examined. Fifty-eight of these were collected in 1959 and the remainder were from various museums. Occipito-nasal lengths, sex, tooth wear and weights have been recorded and used in the reconstruction of information on the life-history. Breeding appears to start in February and terminate in late summer. Most of the present trappings were in the vicinity of the hostel and in the Duttfield-Vaadal area. Mice were never very numerous and appeared to frequent dry-stone dykes and the hostel buildings. The Fair Isle mouse is larger than the mainland mouse and slightly smaller than the St. Kilda mouse.

CONTENTS

	Page
Introduction	439
Material examined	440
Methods	440
Ecology	443
Life cycle	446
Analysis of size	450
Sex ratio	450
Acknowledgments	451
Summary	451
References	452

INTRODUCTION

The Fair Isle field mouse (*Apodemus sylvaticus fridariensis*) was first described (as *Mus sylvaticus fridariensis*) by Kinnear in 1906. In addition to the description, brief reference was made to the situations in which the animal occurred. Miller (1912) elevated it to specific rank and was subsequently supported by Barrett-Hamilton & Hinton (1910–21). More recently Ellerman & Morrison-Scott (1951) have with little justification, made it a subspecies of *A. flavicollis* Melch. whilst Matthews (1952) has favoured a reversion to a subspecies of *A. sylvaticus* L. In 1954, Williamson published a short note on the mice of Fair Isle and in May, 1956, Kikkawa (1959) surveyed their natural habitats. These publications constitute the total work undertaken on this interesting island form.

The following account of the bionomics of *A. sylvaticus fridariensis* is based upon a continuous trapping survey made from late May to the end of October, 1959, and from the beginning of March to the end of May, 1960. Reference has also been made to skulls and skins deposited in various museums. The life cycle and seasonal fluctuations in numbers have been emphasized as, up to the present, lack of material collected over a protracted period has prevented an analysis of this type.

MATERIAL EXAMINED

The following account is based upon the field collection and laboratory examination of fifty-eight animals trapped in 1959 as well as the material obtained by two other collectors.

The Williamson collection consists of fifty-two animals. Of these thirty-eight have been deposited in the Royal Scottish Museum, Edinburgh, eight in the Department of Natural History, University of Aberdeen, and six in the Universitetets Zoologiske Museum, Copenhagen. In the Edinburgh collection three animals were not sexed, two not weighed and fifteen had their skulls broken. One skull was missing from the Copenhagen collection and one animal was not weighed. Although it has not been possible to examine this collection, information on it has been kindly supplied by the Museum. One of the animals was not sexed, one not weighed and four skulls were missing in the material from Aberdeen. Three skins, from the Royal Scottish Museum, collected in June and July, 1955, are without a collector's name. They have, for convenience, been attributed to the Williamson collection.

The Kikkawa collection although originally consisting of fourteen skulls now only appears to contain ten. Eight of these, one of which is broken, are in the British Museum (Natural History) and two in the Bureau of Animal Population, Oxford.

Except for the occasional animal that was badly multilated when trapped, and unsuitable for preparation as a study skin, the Williamson collection represents all the animals caught with no selection of individuals of a particular size or weight (Williamson, private communication). In the account that follows certain inconsistencies may appear in the tabulated data. They can be attributed to the incompleteness of the available information e.g. unsexed animals have to be omitted from Tables 2 to 5 and animals with broken skulls from Tables 4 and 5. Furthermore, as Williamson kept no record of the reproductive state of his animals some of the heavier females listed in Table 3 may have attained their weight as a result of carrying advanced embryos.

METHODS

Longworth traps were used for the trappings in 1959 and 1960 and were continuously set. The occasional trap was sprung without catching anything and periodically rain spoiled the bait (oatmeal). Each mouse was weighed, to the nearest gram, and had its head-and-body and tail lengths measured. It was sexed and notes were made on its reproductive condition. The head was removed and cleaned by *Dermestes* beetles; the occipito-nasal length was measured and the amount of tooth wear recorded.

To obtain a quantitative estimate of tooth wear the following method has been used. If a recently erupted upper tooth row is examined in profile, under a low power monocular microscope, from the inner (buccal) side of the mouth a row of twelve cusps (Fig. 1) is apparent. The notation of these cusps is that adopted by Barrett-Hamilton and Hinton (1910-21). To display them in this way the skull is tilted slightly so that cusp $M^1 1$ is directly opposite

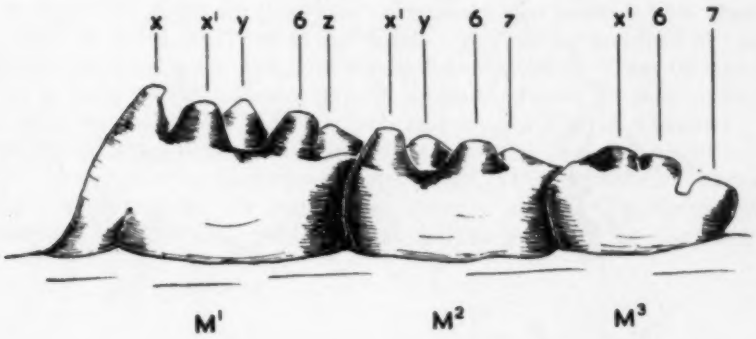

Fig. 1—Inner (buccal) aspect of maxillary tooth row showing full complement of cusps.

M^1x^1. Cusps M^1x, M^1y, M^1z and M^2y represent a row of central cusps whilst M^1x^1, M^16, M^2x^1, M^26, M^27, M^3x^1, M^36 and M^37 represent a row on the inner margin. M^17 was very small and was not apparent as an elevation along the tooth row.

An examination of the 1959 collection showed that the cusps were worn down and eliminated from the row in a fairly regular sequence. M^27 was first to be eliminated and was followed by M^2y. The next to disappear were M^1y and M^1z with M^3x^1 and M^36 becoming a single cusp. The precise order in which these three cusps were lost was variable. Thus by the next phase the following cusps remained: M^1x, M^1x^1, M^16, M^2x^1, M^26, M^3x^1+6 and M^37. Either M^2 or M^3 may first become flattened completely (for convenience referred to as a single cusp) and would be followed by the other. The most worn tooth row examined consisted of M^1x as the only single cusp visible, the rest of M^1 was flattened as well as all of M^2 and M^3.

For convenience, four stages of tooth wear have been recognized, viz:—

Stage 1. All twelve cusps present.

Stage 2. Loss of M^27 or M^27 and M^2y.

Stage 3. Loss of M^27, M^2y and all or any one or two of M^1y, M^1z and M^3x^1+6 *i.e.* a tooth row of 9, 8 or 7 cusps.

Stage 4. Teeth more worn than stage 3.

The method has been found to provide a useful guide to the relative age of an animal.

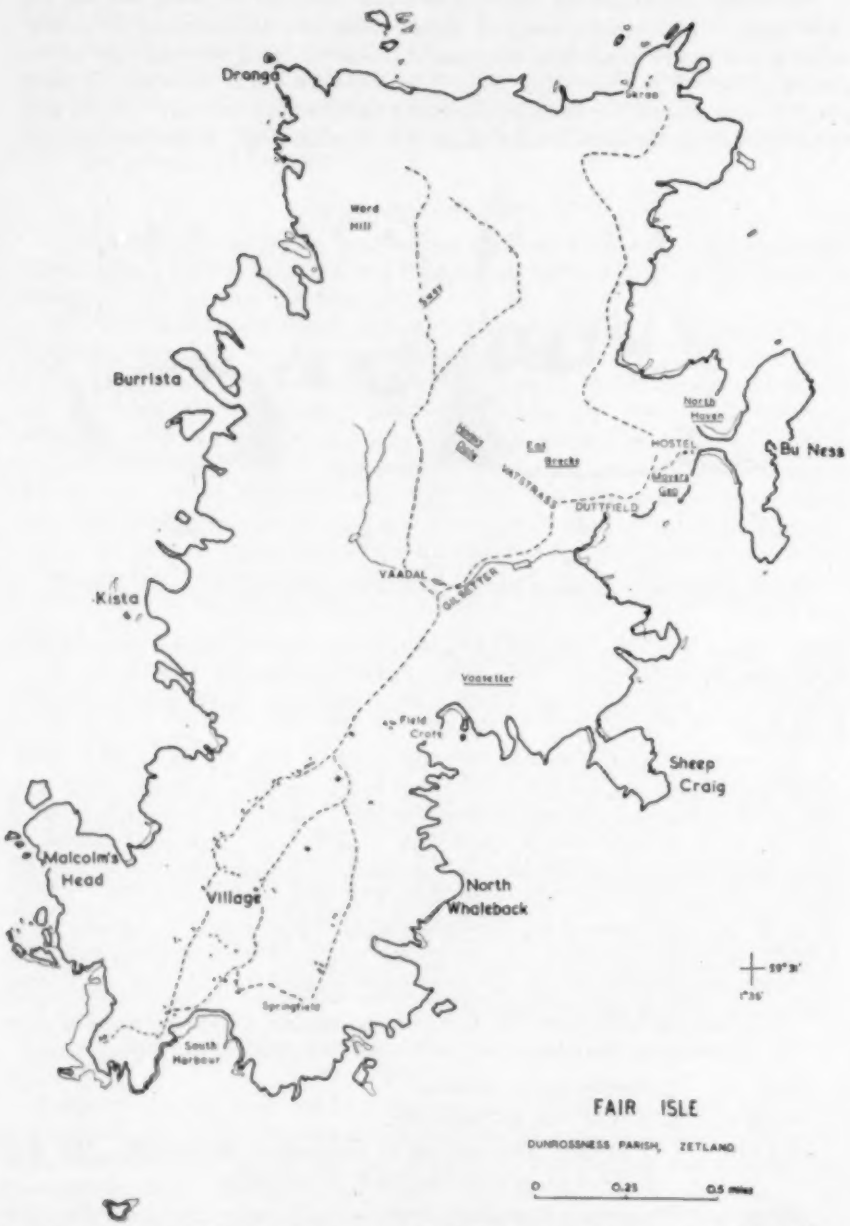

Fig. 2—Map of Fair Isle. In the present survey field-mice have been caught from localities represented by capital letters. The localities from which Kikkawa (1959) caught field-mice have been underlined.

ECOLOGY

Using breakback traps, Williamson caught *Apodemus* at irregular intervals from October 1952 to July 1955. Except for seven animals brought to him from Field Croft his trappings were restricted to the Hostel (Fig. 2) and were primarily undertaken to reduce the numbers of mice which at times became a nuisance. The numbers of mice trapped during each month are thus related to the abundance of the animal. The monthly catches illustrated in Fig. 3 suggest an apparent increase in numbers in October and November.

In May 1956, Kikkawa (1959) set traps in eighteen habitats from Skroo in the north to South Harbour in the south. The survey involved 612 trap-nights and resulted in the capture of twenty-four *Apodemus*. They were caught in ling at Swey and Eas Brecks (Fig. 2), at North Haven, on cliffs at Mavers Geo, in a stone enclosure at Homis Dale and along a dyke at Vaasetter. He concluded that the field-mouse was largely restricted to the northern half of the island and that the main breeding centre was in the ling habitat. Although Kinnear (1906) found the field-mouse common around the crofts during September, 1905, he also caught several specimens among the short ling on

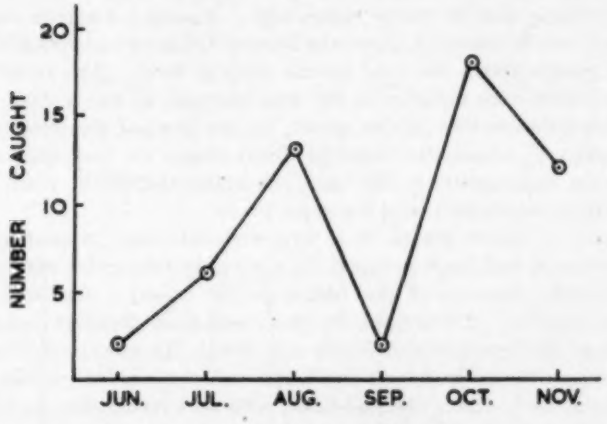

Fig. 3—Monthly catches of field-mice at the Hostel by Williamson, 1952–55.

the higher parts of the island near the north end. Both Kikkawa and Kinnear found the house-mouse (*Mus musculus* L.) restricted to the village and none have been collected in the present survey which has been conducted some distance further north.

The trapping of mice in 1959 and 1960 was almost confined to the vicinity of the Bird Observatory buildings, and to the route followed daily when the permanent bird-traps were visited. Unfortunately, little time could be devoted to trapping in other parts of the island.

The Bird Observatory hostel is at North Haven, in the north-east of the isle, and the trapping area extended south-west from Duttfield, about 400 yards away, to Vaadal, almost a mile distant. A rough dry-stone dyke some five to six feet high and two feet thick starts at the cliff edge at Duttfield, crosses the shallow

valley at Vatstrass, and then follows the north slope of the Gilsetter valley to Vaadal. Most of the mouse traps were set along this wall. The foundations of the dyke are evidently dry and well-creviced, as they are occupied by nesting starlings (*Sturnus vulgaris* L.) and wheatears (*Oenanthe oenanthe* (L.)) as well as by rabbits (*Oryctolagus cuniculus* (L.)) and (presumably) field-mice. The island's main road runs parallel with the dyke, on the north side, and for much of the distance the two are separated only by a grassy ditch or verge some six to ten feet wide. The grass (mainly *Agrostis tenuis* Sibth.) is grazed by sheep and rabbits, and does not generally exceed three to four inches in height. North of the road, the ground at once rises steeply, and the dominant vegetation is ling (*Calluna vulgaris* (L.)), rarely more than six inches tall, with crowberry (*Empetrum nigrum* L.) and prostrate juniper (*Juniperus communis* L.). On the south side of the dyke, the ground is open, with very short ling and grasses. At Duttfield and Vatstrass the land was formerly cultivated, and slopes gently to the Burn of Vatstrass ; at Gilsetter the slope is steeper, and drains to the Burn of Gilsetter, seldom more than fifteen to twenty yards below the dyke. Beyond this stream is an extensive marshy area, probably the bed of a post-glacial lake, dominated by various sedges (mainly *Carex* sp.) and horsetail (*Equisetum palustre* L.) growing nine to twelve inches high. Vaadal is a narrow steep-sided valley to the north of the road, where the Burn of Gilsetter emerges from the hill ground and passes under the road to the marshy level. The remains of an old water-mill here were included in the area trapped, as was a short length of dyke on the south-east side of the marsh, at the head of the deep gully (the Gill of Funniquoy) where the Gilsetter Burn makes its last descent to the sea. The dyke has essentially the same characteristics as the main one, and the neighbouring vegetation is of the same type.

The Hostel at North Haven is a former naval camp, consisting of nine timber or concrete buildings grouped on a roughly triangular acre of closely grazed grassland. Several of the buildings are raised a few inches above ground-level, on piles. A well-built dry-stone wall about five feet high separates the camp from the foreshores of North and South Havens, to the east. The grassland runs to the cliff edge on the north and south sides ; to the west the hill rises immediately above the buildings, with an overhanging rocky outcrop along the northern half. This hill is partly clad with short ling, but is generally grassy. Taller ling occurs at its crest, within 100 yards of the huts. A number of drainage ditches up to three feet deep (generally dry in summer) are in the neighbourhood. A small area of about 120 sq. yards, alongside one building, is enclosed as a garden ; used mainly for growing salad crops (lettuce, radishes, etc.) or flowers. Luxuriant grasses grow around the cultivated beds. Rabbits live under several buildings, and there are two or three large warrens less than 100 yards away. Mouse-traps were set inside and under the buildings, in the garden, along the dry-stone wall and in the nearby ditches. No mice were taken from the ditches, but the other places all provided captures. The interior of the buildings, particularly the kitchen hut, may be invaded by considerable numbers of field-mice from June or July onwards, but these are usually decimated or exterminated before the end of the year, and do not seem to be replaced later in the winter.

Table 1—Results of 1959–60 trappings of *Apodemus sylvaticus fridariensis* on Fair Isle.

Date	Locality	Trap nights (approx.)	No. caught	Percentage success (approx.)
May, 1959	Springfield	70	0	0·0
June, 1959	Hostel	69	3	4·4
	Duttfield/Vaadal	161	3	1·9
	N. & S. Havens	70	0	0·0
July, 1959	Duttfield/Vaadal	217	7	3·2
	Hostel	93	2	2·2
Aug., 1959	Duttfield/Vaadal	217	7	3·2
	Hostel	93	6	9·4
Sept., 1959	Duttfield/Vaadal	105	1	1·0
	Hostel	195	19	9·7
Oct., 1959	Hostel	310	10	3·2
Nov., 1959/Feb., 1960	—	0	0	0·0
Mar., 1960	Hostel	280	0	0·0
Apr., 1960	Hostel	300	0	0·0
May, 1960	Hostel	294	0	0·0

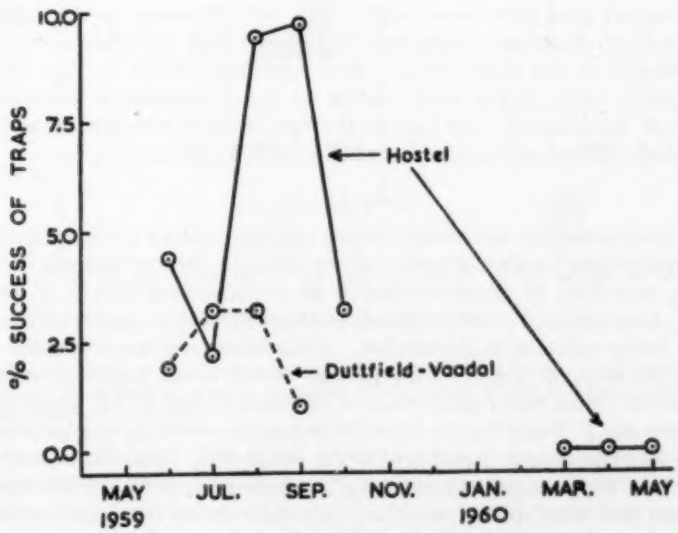

Fig. 4—Trapping success on Fair Isle, 1959–60.

The results of these trappings are summarized in Table 1 and Fig. 4. As the traps were moved infrequently and the animals caught in them permanently removed from the population, care has to be exercised in interpreting fluctuations in population numbers ; particularly as *Apodemus* is apparently at no time very abundant.

No mice were caught at the Hostel between March and May, 1960, although they were caught in each month between June and October, 1959, reaching a peak, as the Williamson collection also indicates, in late summer. This suggests that in this locality a very small population survives the winter and breeds throughout the following summer or alternatively that in spring and summer the area becomes populated by mice from the adjoining moorland which then multiply in their new habitat and are exterminated in the autumn and early winter. As it is subsequently suggested (p. 446) that an appreciable proportion of mice survive the winter, their absence from the traps in this locality from March to May may be attributed to absence and/or very reduced activity during the winter.

In the Duttfield–Vaadal area mice were caught in each month (June to September) that traps were set. Boyd (1959) found *Apodemus* resident in the disused cleitean (dry-stone storage chambers) of Hirta, St Kilda, and it appears probable that a small permanent population is similarly established in the dry-stone dykes in the vicinity of Duttfield and Vaadal on Fair Isle.

Little information is available on the predators of the mouse. The occasional migrant kestrels (*Falco tinnunculus* L.) and short-eared owls (*Asio flammeus* (Pontopp.)) may take a few. The numerous domestic and feral cats probably kill a number whilst dogs are sometimes put to killing mice when a hay or corn-stack is cleared away. It might be supposed that the arctic skuas (*Stercorarius parasiticus* (L.)), which occupy much of the hill ground, would take mice occasionally (they eat lemmings in the far north), but there is no evidence of this despite the fact that P.E.D.'s main work in the summer is on the skua colony. Man must kill a large number each year in the crofts. One of the main checks on the population is probably food shortage as the island is very bare in the four main winter months and much of it probably becomes too waterlogged for mice to live in.

LIFE CYCLE

The data on weights and occipito-nasal lengths (Tables 2 to 5 and Fig. 5) throw considerable light on the life cycle of the animal. Young animals, i.e. those weighing less than 25 grams or having an occipito-nasal length of less than 26 mm., were collected in all months from May to October, with their maximum number being collected in September. (This latter statement is made on the basis of the animals caught in the present survey when trappings were made each month. Bias could result from Williamson's data as his trappings were less systematic.) From May to June the population contains a large percentage of older animals (stages 3 and 4 of tooth wear) (Fig. 5) and it is only subsequently that the younger animals occur in appreciable numbers. Furthermore, it appears that some of the previous year's animals survive into October, and if the male and female in the November histograms (Fig. 5) with a stage 3

tooth-wear are regarded as from the previous years' stock, then some survive into November.

Lowe (1957) has suggested that in captivity *A. s. sylvaticus* L. has a gestation period of twenty-six days. The animals of a litter reared in captivity by Delany (unpublished results) attained a weight of 10 grams after four to five weeks and 15 grams after about eight weeks. If this time scale is applied to two young mice, each weighing 14·4 grams, caught on Fair Isle on 18th and 19th May, 1956, it is estimated that they were born in late March and conceived in late February. This, from the data available, represents the earliest

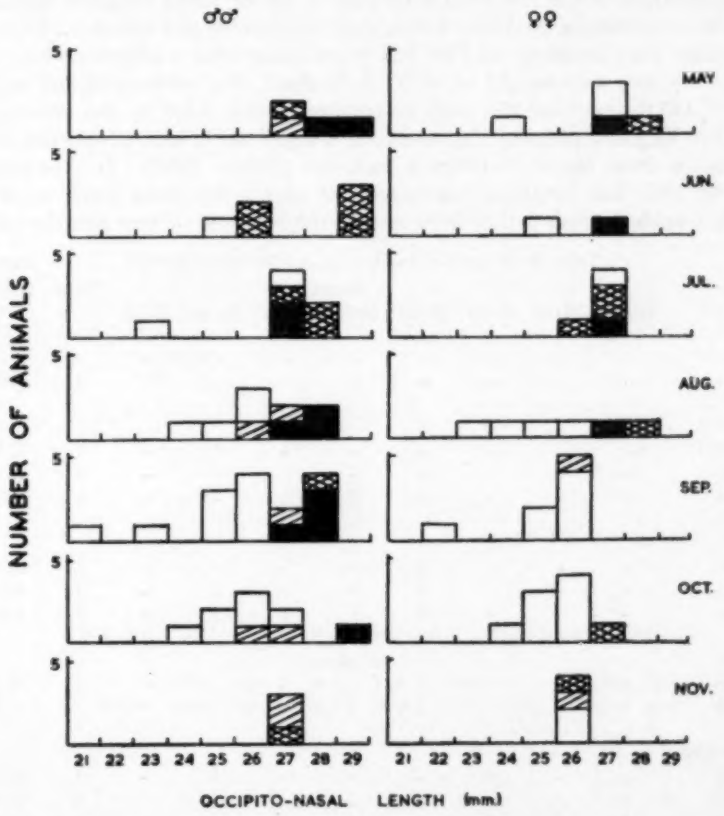

Fig. 5—Occipito-nasal lengths of field-mouse skulls according to month of capture. Stage of tooth wear is also included ; stage 1, unshaded blocks ; stage 2, diagonal lines ; stage 3, cross hatched and stage 4, solid black.

breeding on Fair Isle. The latest births appear to be in early September. The large catches from August to October suggest the peak period of births to be from early June to late August. In which case, the breeding population is probably largely derived from animals surviving the previous winter.

Information on breeding from pregnant and lactating females is scant. Of the twenty-three females caught between June and October, 1959, only one was pregnant. This was caught on August 15th and contained four very advanced embryos. Another female caught the same day was lactating. Two others were lactating when caught on the 6th and 10th July. The absence of lactating females in a very small September sample suggests that by that time most breeding has ceased. Of the eleven females Kikkawa (1959) examined in May five were pregnant and three lactating. The only record of the number of embryos was from one female containing five.

Comparison of the life cycle with that of the St Kilda mouse is hampered by lack of records for the latter during July, September and October. However, it appears that breeding on Fair Isle takes place over a longer period, there being no young mice caught on St Kilda in April, May or the first half of June (Boyd, 1959) and that the peak is reached rather later in the season. In southern England pregnant females form a consistently high proportion of the population from March to October inclusive (Baker, 1930). It thus appears that on Fair Isle breeding commences at about the same time as further south, reaches a peak rather later and terminates one or two months earlier.

Table 2—Weights of males of *A. s. fridariensis* (Kinn.)

Date	Grams.								Total	Collector
	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49		
May										
1954	–	—	–	–	–	–	–	–	–	—
1956	–	–	–	–	5	1	1	1	8	J.K.
June										
1954	–	–	–	–	–	–	1	–	1	K.W.
1955	–	–	–	–	–	–	1	–	1	K.W.
1959	–	–	–	2	1	1	–	–	4	P.E.D.
July										
1953	–	–	–	1	–	–	–	–	1	K.W.
1955	–	–	–	1	–	–	–	–	1	K.W.
1959	–	1	–	2	–	1	2	–	6	P.E.D.
August										
1953	–	–	–	2	–	1	–	–	3	K.W.
1959	–	1	–	2	2	2	–	–	7	P.E.D.
September										
1953	–	–	–	–	–	–	1	–	1	K.W.
1954	–	–	–	–	1	–	1	–	2	K.W.
1959	1	1	5	2	2	1	1	–	13	P.E.D.
October										
1952	–	–	1	2	–	–	–	–	3	K.W.
1953	–	–	–	2	1	1	–	–	4	K.W.
1959	–	3	2	–	–	–	–	–	5	P.E.D.
November										
1952	–	–	–	–	1	–	–	–	1	K.W.
1953	–	–	–	2	3	–	1	–	6	K.W.

Table 3—Weights of females of *A. s. fridariensis* (Kinn.)

Date	Grams								Total	Collector
	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49		
May										
1954	–	–	–	1	–	–	–	–	1	K.W.
1956	2	–	–	–	4*	1	–	–	7	J.K.
June										
1954	–	–	–	–	–	–	–	–	–	—
1955	–	–	–	–	–	–	–	–	–	—
1956	–	–	1	–	1	–	–	–	2	P.E.D.
July										
1953	–	–	–	–	–	–	–	–	–	—
1955	–	–	–	–	–	1	–	–	1	K.W.
1959	–	–	–	–	2	1	–	–	3	P.E.D.
August										
1953	–	–	–	–	–	1	–	1	2	K.W.
1959	–	2	1	1*	1	1	–	–	6	P.E.D.
September										
1953	–	–	–	–	–	–	–	–	–	—
1954	–	–	1	–	–	1	–	–	2	K.W.
1959	1	–	4	2	–	–	–	–	7	P.E.D.
October										
1952	–	1	7	2	–	1	–	–	11	K.W.
1953	–	–	–	–	1	–	–	–	1	K.W.
1959	1	3	–	1	–	–	–	–	5	P.E.D.
November										
1952	–	–	2	1	–	–	–	–	3	K.W.
1953	–	–	1	–	–	–	–	–	1	K.W.

* One pregnant.

Table 4—Occipito-nasal lengths of males of *A. s. fridariensis* (Kinn.)

	Length (mm.)									
	21·0–21·9	22·0–22·9	23·0–23·9	24·0–24·9	25·0–25·9	26·0–26·9	27·0–27·9	28·0–28·9	29·0–29·9	Total
May	–	–	–	–	–	–	1	2	2	5
June	–	–	–	–	1	2	–	–	3	6
July	–	–	1	–	–	–	4	2	–	7
August	–	–	–	1	1	3	2	2	–	9
September	1	–	1	–	3	4	2	4	–	15
October	–	–	–	1	2	3	3	–	1	10
November	–	–	–	–	–	1	5	–	–	6

Table 5—Occipito-nasal lengths of females of *A. s. fridariensis* (Kinn.)

	Length (mm.)									
	21·0–21·9	22·0–22·9	23·0–23·9	24·0–24·9	25·0–25·9	26·0–26·9	27·0–27·9	28·0–28·9	29·0–29·9	Total
May	–	–	–	1	–	–	6	1	–	8
June	–	–	–	–	1	–	1	–	–	2
July	–	–	–	–	–	1	4	–	–	5
August	–	–	1	1	1	1	1	1	–	6
September	–	1	–	–	2	5	–	–	–	8
October	–	–	–	1	3	4	2	–	–	10
November	–	–	–	–	–	4	–	–	–	4

The data in Tables 4 and 5 are based upon fifty-eight animals collected by Davis, forty-one by Williamson and twelve by Kikkawa.

ANALYSIS OF SIZE

The weights of *A. s. fridariensis* (Tables 2 and 3) suggest that it is appreciably larger than *A. s. sylvaticus*. Evans (1942) and Miller (1958) in surveys extending over ten and eight months of the year respectively, found a very small percentage of animals weighing more than 25 grams. From the same area (Berkshire) the heaviest animal trapped by Southern (Boyd, 1959) was a male weighing 32 grams. On Fair Isle mice frequently weigh more than 30 grams. Boyd (1959) found numerous males of *A. s. hirtensis* weighing 46–50 grams and a few of 51–55 grams. *A. s. fridariensis* never attains either of these weight classes, the largest individuals weighing 45 grams.

On both Fair Isle and St Kilda (Boyd, 1959) females do not in general attain as great a weight as males. Boyd (1959) attributed this, in part, to the variation in weight of the male reproductive organs. However, examination of the occipito-nasal lengths, suggest that the largest males are bigger than the fully grown females. The maximum length of the male skull was 29·1 mm. and of the female, 28·2 mm.

SEX RATIO

In estimating sex ratios cognizance has been taken of the two trapping methods adopted as one type of trap may be more successful in catching one sex. As Kikkawa (1959) used both breakback and Longworth traps his figures have been omitted from Table 6. Both types of traps with one exception caught more males than females, although the overall disparity between the two sexes is not great. The slightly higher incidence of males in the Longworth traps can probably be accounted for by increased wandering and foraging activity of the males. There is little evidence of the numbers of one sex being disproportionately large.

Table 6—Sex ratios of *A. s. fridariensis* (Kinn.)

Date	Longworth traps Number	Ratio ♂/♀	Breakback traps Number	Ratio ♂/♀
May–June	6	2·00	3	2·00
July–August	22	1·44	9	1·25
September–October	30	1·50	26	0·73
November–December	—	—	11	1·75
	58	Av. 1·52	49	Av. 1·04

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SUMMARY

1. Fifty-eight specimens of the Fair Isle field-mouse (*Apodemus sylvaticus fridariensis* (Kinnear)) were trapped in 1959. These, together with fifty-two animals caught by Williamson from 1952 to 1955 and ten caught by Kikkawa in May, 1956, have provided the material on which the paper is based.

2. The weight, occipito-nasal length, sex and tooth wear of the upper molars are recorded, as far as possible, for each animal. The amount of tooth wear has been determined by counting the number of cusps eliminated from the tooth row. This has been used as an index of age.

3. The main trapping localities were along the dry-stone dykes in the Duttfield–Vaadal area and at the bird observatory hostel. Both produced catches from June to September, when the last traps were set in the former locality, and in October from around the hostel. In both localities the populations were not high although a sharp increase in numbers occurred at the hostel in September and October. In the vicinity of the dyke the vegetation consists mainly of grazed grassland and short ling. Around the hostel buildings is a mixture of stone-dykes, grassland, garden and ling moorland. No animals were caught at the hostel between March and May, 1960.

4. No precise information is available on predators. Possibilities include migrant kestrels and short-eared owls as well as cats, dogs, skuas and man. Sparsity of food in winter will also probably restrict numbers.

5. Breeding probably starts in late February, reaches a peak between June and August and terminates in September. A number of animals survive a second summer.

6. In size, the Fair Isle mouse is intermediate between those of the mainland and St Kilda.

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THE SOURCES AND NATURE OF SALIVARY SECRETIONS IN AMPHIBIA

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(With 2 plates and 1 figure in the text)

The presence of an amylase in the saliva of the frog and toad has been confirmed and is reported for the first time from the frog's tadpole and adult *Xenopus*, *Salamander* and *Triturus*. Proteases have also been demonstrated in the buccal secretions of the frog and salamander. An attempt has been made to identify histologically the cells responsible for the secretion of these enzymes. The relation between the enzyme secreting tissues and the ciliary tracts on the amphibian palate is described and their possible functional significance is discussed.

CONTENTS

	Page
Introduction	453
Material	454
Methods	454
Amylase determination	454
Qualitative	454
Quantitative	455
Protease determination	455
Cytological	456
Results	456
Enzyme determinations	456
Amylases	456
Qualitative	456
Quantitative	457
Proteases	458
Cytological investigation	459
Introduction	459
Intermaxillary glands	461
Glands of the tongue	465
Buccal epithelium of *Xenopus*	468
Ciliary tracts of the palate	469
General resumé and discussion	471
Summary	473
Acknowledgments	474
References	474
Explanation of plates	475

INTRODUCTION

Following the confirmation by Francis & Eisa (1951) of the announcements by Lépine (1870) and Junold (1933) that the saliva of the frog contains a diastatic ferment, the investigation has been extended with the object of determining more precisely the nature of this enzyme and the extent to which

it occurs in other Amphibia. At the same time glandular tissues associated with the mouth of the frog and other Amphibia have been studied by various histological and cytological techniques. This enquiry has led to the additional discovery of a protease in the amphibian saliva.

MATERIAL

The following animals were used for both physiological and cytological study :—

SALIENTIA.

The common frog (*Rana temporaria temporaria*) adult and tadpole stages.
The common toad (*Bufo bufo bufo*) adults.
The South African clawed toad (*Xenopus laevis*) adults.

CAUDATA.

The palmate newt (*Triturus helveticus*) adults.
The warty newt (*Triturus cristatus cristatus*) adults.
The spotted salamander (*Salamandra salamandra salamandra*) adults.

METHODS

Amylase determination

Qualitative

Samples of larval saliva were collected by inserting the glass cannula of a tuberculin syringe into the tadpole's mouth and flushing the pharynx with 0·01 ml. of saline which was then recovered by sucking back into the syringe chamber. Each tadpole was flushed twice and the accumulated washings from 100 individuals stored for enzyme estimations.

Saliva from later stages (immediately prior to metamorphosis and older) was secured by swabbing with saline-soaked cotton-wool pads mounted on either stainless steel wire (for adults) or glass rods (for tadpoles). Saliva was extracted from the pads by soaking for one hour in 2 ml. saline and then draining. Usually twenty adults were used for each experiment and when this number of animals was not available, each animal was swabbed twice. Any swabs accidentally blood-contaminated were rejected.

Bacteriological tests showed that starch-splitting bacteria were present in extracts obtained by both methods and that these were not killed by toluene (used as bactericide by Francis & Eisa, 1951). After several trials it was found that penicillin and streptomycin used together effectively kill all such organisms. Throughout the present series of experiments, therefore, two drops of a solution of each of these antibiotics (penicillin, 1650 i.u./ml. and streptomycin, 10,000 i.u./ml.) were added to each culture tube. One further precaution proved necessary, namely, each fresh batch of penicillin and streptomycin had to be tested since some samples were found, of themselves, to give positive reactions with the Benedict's solution used for showing the presence of reducing sugars. Controls to check this were accordingly set up with every experiment.

Enzymic activity of the saliva was determined by Benedict's test for reducing sugar. 0·5 ml. of the extract was incubated with 1 ml. 1 per cent

starch solution and two drops each of penicillin and streptomycin. Substrate activity was checked by substituting saline for extract in the above mixture whilst the effectiveness of the antibiotics was estimated by incubating 0·5 ml. saline with two drops of penicillin in one tube, two drops of streptomycin in a second, and two drops of both in the third. A parallel series of tubes in which the starch was replaced by glycogen, was also employed. Incubation, in all instances was at 37°C and for about 24 hours.

Quantitative

In 1952 van Loon, Likins & Seger published a colourimetric method for estimating the quantity of amylase present in blood serum and this technique has been adapted to give comparison between the amylase present in the mucosae of the tongues and of the intermaxillary glands of the frog, toad and salamander. The procedure is as follows :— the mucosal layer of the tongue of a pithed animal was carefully stripped from the under-lying muscle, blood vessels, etc., blotted on filter paper, and weighed on a torsion balance. (Four to six tongues yield ample material for one experiment). The mucosae were then completely homogenized in 2 ml. saline (0·75 per cent). After dilution (1 : 10) with further saline the suspension was centrifuged for half-an-hour at 5,000 r.p.m. to precipitate the cell debris and the decanted supernatant fluid was then treated in exactly the same way as van Loon *et al.* (1952) treated their diluted blood serum. Intermaxillary glands were also removed and treated in the same way. No quantitative tests were made on tadpoles.

Protease determination

The results of an histological and cytological investigation described below, suggested the presence of a protease as well as an amylase in the frog's saliva, and further that such an enzyme, if indeed it should prove to be present, would most likely originate in the intermaxillary gland.

Preliminary tests, made by incubating casein (Hammarsten's) with salivary extracts obtained either by lavage or swabbing, gave weakly positive results when tested with Folin-Ciocalteau's reagent (*vide infra*) but a similar test with an homogenate of the intermaxillary gland gave much more definite results.

In order to obtain tissue extracts of a convenient strength, pithed frogs were pinned through the angles of the mouth so as to prop open the lower jaw ; the snout of each was then skinned and the intermaxillary gland removed and placed in a moist chamber until thirty-six glands had been accumulated. After weighing on a torsion balance and homogenizing with a glass homogenizer in 2 ml. saline (0·75 per cent) as above, the homogenate was strained through a linen gauze and diluted with saline to give the volume required for the particular experiment, the concentration being somewhere between 14 and 20 mg. per ml. (The average weight of one gland is 3·42 mg.)

Two tubes were set up as follows :—

Tube A (control) containing 3·5 ml. extract (boiled for five minutes) ; 5·25 ml. casein ; 5·25 ml. buffer at pH 6·0 (previously determined as giving optimum results).

Tube B containing 3·5 ml. extract ; 5·25 ml. casein ; 5·25 ml. buffer at pH 6·0.

Both these tubes were incubated together at 40°C and aliquot samples of 2 ml. were withdrawn from each at zero time and then at half-hourly intervals up to three hours. To each sample was added immediately 0·75 ml. trichloracetic acid (five per cent soln.). It was then centrifuged for two minutes at 1750 r.p.m., the supernatant fluid decanted into a test-tube, and 4 ml. of sodium carbonate (4·6 per cent aqueous soln.) added. Finally, after the addition of 2 ml. Folin-Ciocalteau reagent (E. Gurr) the colour was allowed to develop for thirty minutes at room temperature. The transmission of light at 760 mμ was then read on a Unicam ' 600 ' spectrophotometer.

Paper chromatograms were prepared using standard procedures. Propanol water (4 : 1) was used as a solvent and, after running overnight, the paper was dried and the spots developed by spraying with Ninhydrin (0·25 per cent in water saturated butanol) at 80°C.

Cytological

For the cytological part of the work uniform results from one sample to another and one species to another was obviously essential. Various fixatives and stains were tried and finally PAS with fast green F.C.F. counterstain following fixation in Bouin's fluid (aqueous) was adopted as routine. The timing of each operation was accurately controlled so as to secure uniformity. Piccolyte (in xylol) was used as the mounting medium.

Some additional sections were stained in an Azure A (MacNeal) and others in Sudan Black B.

RESULTS

Enzyme Determinations

Amylases

Qualitative

Frog and Toad—adult

Repetition of the experiments already reported (Francis & Eisa, 1951) but using penicillin and streptomycin to control bacterial activity, entirely confirmed the earlier results that the saliva contains a diastatic ferment. One further test was applied to determine whether the enzymic activity was that of an amylase rather than a phosphorylase. The extract from the frog's oral tissues was incubated with glucose monophosphate with negative results. The entire behaviour of the enzyme, and in particular its behaviour on the paper chromatograph reported by Francis & Eisa, 1951, therefore, suggests that it is a true α-amylase.

Frog, larval stages

The present study has been limited to the tadpoles of *Rana* at all ages from seventeen days after hatching (early internal gill stage) to young frogs, and of *Bufo* (internal gill stage only) over several seasons and, provided that the appropriate method of collection was used positive results were obtained. It is clear then that at least some Amphibia possess buccal diastases in both larval and adult stages*.

* Junold (1933) investigated the diastatic activity of the buccal epithelium of the larvae of *Pelobates fuscus* and came to the conclusion that the saliva of the premetamorphic stages of these animals possessed no diastatic activity.

Other Amphibia

Of the remaining four species of Amphibia studied, only the adults were examined and all of them gave positive results.

Quantitative

The methods employed for collecting the saliva preclude a satisfactory quantitative comparison, on an objective basis, of the relative amounts of enzyme present in the several amphibian species, but homogenates of the mucosa from the upper surface of the tongue of the frog, toad and salamander and of the intermaxillary glands of the same animals and of *Xenopus* yielded the following results when estimated for amylase by the method of van Loon *et al.* (1952).

Table 1—Results of quantitative experiments for the presence of amylase in the tongues and intermaxillary glands of various amphibia expressed as the mean ± standard error, except for the intermaxillary glands of the toad and salamander where observations are too few to permit statistical treatment.

Tissue	Animal	Amylase units per gram wet weight	No. of observations	Amylase units per organ
Tongue	Frog	4·182±0·606	13	0·475 ±0·071
	Toad	2·599±0·535	8	0·251 ±0·0086
	Salamander	0·97 ±0·405	9	0·0459±0·0056
Inter-maxilliary gland	Frog	6·937±0·748	7	0·02 ±0·0028
	Toad	mean 10·5	2	mean 0·031
	Xenopus	6·66 ±1·268	8	0·035 ±0·0059
	Salamander	mean 9·116	3	mean 0·0183
Sub-maxillary gland	Male mouse	62·74±5·654	6	5·294 ±1·11

It must be noted that although the concentration of amylase per gram wet weight is much higher in the inter-maxillary glands than in the tongues yet, because of the greater mass of the latter organs, the total quantity of enzymes contributed to the saliva from each of these two sources is in the reverse order. Thus the difference between amylase concentration in the salamander tongue and that in the intermaxillary gland estimated per gram wet weight is highly significant ($P=0.001$) but estimated as amount of amylase per organ it is not ($P=0.2$). The difference between the frog's tongue and salamander's tongue, on the other hand is highly significant ($P=0.001$) by both methods of estimation. The difference between the intermaxillary gland and the tongue of the frog on the whole organ basis is highly significant ($P=0.001$) but on the wet weight basis it is less so ($P=0.05$). There are obviously no significant differences between the inter-maxillary glands of any of the amphibia studied. Finally the concentration of amylase per gram wet weight in the frog's tissues is only about one tenth as great as in the mouse's submaxillary gland.

Junold (1933) attempted a quantitative estimation of amylolytic activity by taking " pieces " of buccal mucosa, inter-maxillary gland, tongue, liver and muscle from *Rana esculenta*, *R. temporaria* and *Bufo bufo* and incubating each piece of tissue (apparently whole) with starch substrate for various lengths of time at 38–40°C. then estimating the amount of sugar released. Junold, however, did not apparently think it necessary to weigh the samples of tissues he used and hence invalidated his whole experiment.

Proteases

The results of protease determinations are best shown by means of a graph drawn from the results of a typical experiment (Fig. 1), the presence of tyrosine and tryptophane was demonstrated chromatographically. It will be seen that

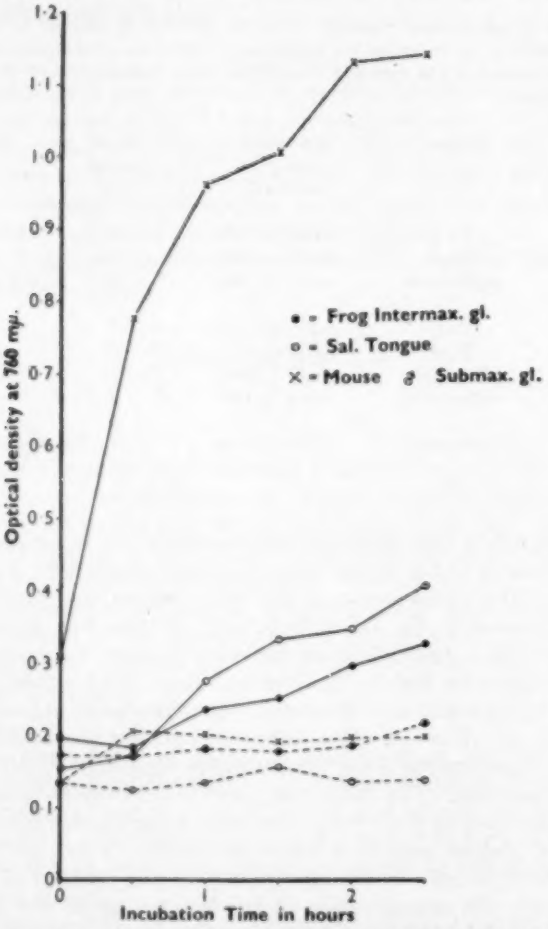

Fig. 1—Graph representing a typical series of protease determinations using Folin-Ciocalteau's reagent to estimate amino-acids released from casein substrate ; broken line=controls.

the optimum time for the experiment when the extract is incubated at 40°C is about two to three hours when the maximum difference between control and experimental tubes is recorded.

These results are comparable with those obtained from extracts made from the submaxillary glands of the male mouse when the extract is of comparable strength (i.e. weight of tissue per unit volume of saline).

Extracts of the frog's tongue are completely negative for protease. The toad's tongue, on the other hand, shows a slight positive reaction when tested with Folin-Ciocalteau but no trace of amino-acids could be detected on the paper chromatograms and it was concluded that the slight reaction with Folin-Ciocalteau was due to some substance other than amino-acids.

The salamander's tongue proved quite definitely positive with Folin-Ciocalteau and also by paper chromatography (Fig. 1). Newt tongues were too small and too few to allow satisfactory protease determinations, and for the same reason the intermaxillary glands of urodeles have not been tested.

Cytological investigation

Introduction

The possibility of a variation in the quantity of enzymes present in the tissues of various species naturally led to a consideration of the site or sites occupied by the glandular tissue responsible for their production. Lépine (1870) appears to be of the opinion that the mucosa of the tongue is the main source of amylase, while Junold (1933) and Francis & Eisa (1951) sampled several tissues—intermaxillary gland, buccal mucosa, tongue—and got positive results in each case. Nevertheless, it is evident that an enzyme contained in the saliva which bathes the whole tongue and buccal mucosa will contaminate these tissues and may give a positive titre even though the tissues concerned may not themselves produce the enzyme. The intermaxillary gland, situated as it is well outside the mouth cavity proper, is much less likely to be contaminated in this manner, though even here such a possibility cannot be ruled out entirely.

It is known that proteases are associated with the cells in the terminal portion of the intracellular ducts and amylase with the glands of the acini of the submaxillary glands of the male mouse, cf. Lacassagne (1940) Fekete (1941), Junquiera *et al.* (1949), Junquiera (1951) and Junquiera & Hirsch (1956). The cytological picture presented by these two regions is quite different. Cells in both produce granular secretions but those in the terminal tubules are PAS negative (staining green with the counterstain) but staining blue with Azur A, as also do the luminal contents. In the deeper parts of the cell, towards the nucleus, a slight metachromatic (mauve to purple) reticulum can be seen under the oil immersion around the granules. The acinar cells, however, show with the PAS/fast green technique a dense purple reticulum (diastase and hyaluronidase stable) the meshes of which are filled by a green (in the deeper parts of the cell) or colourless (towards the luminal border) material. With Azur A a metachromatic (mauve) reticulum surrounds colourless meshes. A slight amount of material stainable with Sudan Black B is present in the acini while the luminal contents also take a grey hue in places.

This picture has been taken as a standard against which to assess the amphibian material.

Little study has been made of the histology of the amphibian glands and virtually none of their cytology. Wiedersheim (1876) regards the intermaxillary gland as a true mucous gland. Seifert (1932), who gives a detailed account of the disposition of special buccal glands in caudate amphibia, questions this conclusion on histological grounds and rightly points out that we cannot, as is generally assumed, class all the buccal glands of Amphibia together, there being, for example, histological differences between the intermaxillary gland and those of the tongue. He regards the question of whether the intermaxillary gland should be regarded as mucous, serous or mixed as *sub-judice*, but says that the palatal glands of *Eurycea guttolineata* are strictly comparable in appearance (viz. partly mucoid, partly granular) to the mixed salivary glands of mammals. Apart from noting the staining reactions of the intracellular granules of the intermaxillary gland to some three or four histological stains, Seifert confined his attention to the topography and gross anatomical relations of the several concentrations of glandular tissue in some thirty-six species of caudate Amphibia. E. Müller (1932) deals in a similar way with a very wide range of salientian species (forty-three in all) mentioning the histological aspects only in the most summary manner. Similar remarks could apply to the description of these glands in Ecker's & Wiedersheim's (1904) revision of Gaupp's *Anatomie des Frosches*. Noble (1931) suggested (p. 201) that "The chief function of all these glands would seem to be to render the tongue more adhesive." This would appear unlikely in view of the fact that they are also present in those toads which lack a tongue. Noble was in error when he believed that the intermaxillary gland as well as the tongue is absent in Pipidae (p. 202)—at least it is present and well developed in *Xenopus* (see below p. 463). Attempts by the present author to change the viscosity of frog's mucus obtained from the skin by means of intermaxillary gland secretion gave only negative results.

Examination of the oral tissues—buccal mucosa, tongue, intermaxillary and post-choanal glands—of the frog shows that at least three types of secretory products can be identified. These are, *mucin*, staining reddish purple with PAS appearing in the early stages of its secretion as a reticulum and in the later stages filling the rather broad goblets completely before its final discharge ; *PAS positive material*, resistant to both diastase and hyaluronidase, which in its early stages is also reticular but may finally become granular (in some parts of the tongue of frog) or retain its reticular form throughout (tongue of toad). The colour reaction with PAS of this secretion is always distinctly bluer than that of the true mucin. *PAS negative granules*, which although originating near the nucleus eventually lie in a goblet-like recess. It is possible that a non-stainable secretion is also produced—see below under salamander's tongue.

In general, the cells of the intermaxillary glands of the frog and toad may be compared with those of the terminal portions of the intralobular ducts of the male mouse and the cells lining the crypts of the amphibian tongue with the granule secreting cells of the salivary acini in the male mouse. Nevertheless,

differences in detail must be noted. For example, the granules in the salivary acini stain green only in the deeper parts of the cell, becoming colourless by the time they reach the luminal border whereas in the amphibian tongue they stain both with fast green and Azur A. The appearance of the purely mucous gland cells (in the retro-lingual gland of the mouse and on the underside of the frog's tongue) shows that the PAS positive material in the salivary acini and in the lingual crypt differs from the mucus in its behaviour towards PAS.

Intermaxillary glands

The *intermaxillary glands* and palatal glands of Amphibia are all tubular glands which open by ciliated openings on to the palate. There is some variation in the number of tubules per opening but in all cases the collecting ducts are short ; most of the cells comprising the glands represent active glandular tissue.

Rana temporaria (adult)

In *Rana temporaria* the ducts of the intermaxillary gland discharge their secretion into some five or six pits on the palate just anterior to the vomerine teeth (Müller's Type II), see Pl. 2, fig. 11. The epithelium lining the openings, and the ducts for a short distance deep to them, is ciliated ; otherwise the whole tubule is lined with columnar cells with basal nuclei. A few mucin goblet cells occur near the openings of the tubules amongst the ciliated epithelum, but apart from these the entire glandular tissue appears to produce only the granules which are PAS negative (Pl. 1, figs. 1 & 3) and which, as judged by this and their reactions to Azur A, Sudan Black B as well as routine stains (such as Mallory's ; haematoxylin and eosin ; Masson's trichrome) appear to be identical with the granules produced by the terminal portions of the intra-lobular ducts of the submaxillary glands of the male mouse (Pl. 1, fig. 5).

The cells of the *palatal glands* are not identical with those of the intermaxillary gland. Their cytoplasm takes a distinct pinkish tinge with PAS, especially towards the base of the cell, while the granules, lying towards the luminal border, are finer, less distinct and more bluish in tone than those of the intermaxillary gland. Cells of very similar type, with pinkish cytoplasm have been noticed in the deeper parts of the mouse submaxillary gland.

Rana temporaria (*larval development*)

The primordium of the gland appears very rapidly at about the time of metamorphosis of the tadpole, about the time the hind-legs stage is reached, a fact first noted by Oeder (1905, 1906). There is, however, considerable variability, and time in days from hatching can be only a very approximate measure of the degree of development attained by any tadpole. Amylases can nevertheless be experimentally demonstrated in the mouth of tadpoles prior to this stage ; protease tests have not been applied.

Examination of transverse sections of the heads of a close series of tadpoles taken every other day from about thirty-six days until sixty-six days after hatching, and then at two months after metamorphosis shows that, coincident with the development of the intermaxillary gland, a profound change occurs in

the character of the buccal epithelium. Prior to the onset of development of the intermaxillary gland the roof of the mouth of the tadpole anterior to the pharynx is covered by a single, or at most double layer of epithelium. The deeper layers of cells (where two layers occur) consists of cubical epithelium with centrally placed large nuclei and little cytoplasm, the superficial layer (which may be the only layer), on the other hand, consists of flattened squamous cells, the centrally placed nucleus of each forming a bulge in the outline when they are seen in transverse section. The whole of the superficial cytoplasm of this layer is peppered with fine granules which also cover the free surface of the cells and which presumably, therefore, pass through the cell membrane in granular form. These granules are differentially stained by PAS and fast green ; some are green and some are purple. It is not always easy to distinguish both types by direct examination, since the strong purple colour tends to mask the paler green but by the use of colour filters the presence of both types can be demonstrated. Thus, the buccal mucosa of the tadpole may be regarded as bi-potential (or perhaps better as pleuri-potential) any cell of which may produce granules which may react either positively or negatively to PAS. One does not find true mucin producing cells of the usual goblet type in the mouth proper at this stage although they are present in the pharynx and oesophagus.

The first indication of true mucous glands can be seen a little later than the first appearance of the intermaxillary gland as small red-staining, spherical cells lying amonst the stratified epithelium. Within about four days these have increased in size and have become filled with red granules. Small red-staining cells have also appeared in the mucosa of the tongue by this stage. These PAS positive cells of both palate and tongue gradually increase in size and number and at the same time migrate towards the surface of the mucosa so that within about four weeks after their first appearance many have become transformed into typical goblet cells of the mucoid type. At about the same stage the surface of the tongue is already folding to form crypts and the cells lining these are beginning to develop PAS positive granules characteristic of the adult tongue.

By the same stage, i.e. with four legs and a tail, just prior to metamorphosis, the intermaxillary gland has attained a histological and cytological differentiation comparable with that of the adult, so that during the last twenty-eight days or so of larval life the buccal epithelium of the frog's tadpole becomes transformed from a simple secretory epithelium comprising cells capable of producing both PAS positive and PAS negative material to a stratified epithelium comprising several types of cells none of which produces a granular secretion. The PAS positive material is now mucin and is produced only by goblet cells, while the PAS negative material originates in specialized glandular tissue proliferated from the mucosa but lying wholly deep to it—the intermaxillary gland.

Although some three or four weeks may be required for the complete transformation as outlined above, yet the buccal mucosa loses its capacity to secrete the PAS positive and PAS negative granules very rapidly (within three or four days) after the intermaxillary gland has commenced to develop.

The first sign of this gland appears at about the same time as the hind limbs as a pair of lateral buds beneath the nasal capsules (Pl. 2, fig. 9). At these sites the cells of the superficial layer of epithelium become more cubical and merge with those of the deep layer as both begin actively to proliferate and produce buds projecting into the sub-mucosa. Some two days later PAS negative granules can be seen in some of the cells of the developing gland, although the cells concerned have not yet attained their full differentiation. Nevertheless, this differentiation is achieved within the first five or six days of their development. There is, therefore, only a very short interval—a day or two at most—during which PAS negative granules are not being liberated into the mouth cavity from the buccal epithelium or from a gland derived from it, whereas, there is an interval of nearly three weeks between the disappearance of PAS positive granules from the cells of the buccal epithelium and the achievement of functional maturity by the cells producing PAS positive granules on the surface of the tongue.

There is, therefore, a short gap in the production of PAS negative granules which, assuming that these granules may be correlated with enzyme production, should give a stage at which one could get consistently negative results. In any one hundred tadpoles, of the same age (say forty-five days) however, there will be so much variation in development that a purely negative result hardly ever occurs. There may be, however, much variation in the strength of the enzyme reaction from one sample to the next. With the swabbing method a better yield of secretion per sample results and the inconsistency is less apparent.

Bufo bufo

The intermaxillary gland of the adult toad (*Bufo bufo bufo*) shows no noteworthy cytological difference from that of the frog, but Müller (1932) regards it as highly specialized morphologically (Type V against Type II for frogs). Only one pair of slit-like openings occurs, lying just anteromesial to the internal nares. The buccal mucosa, posterior to the openings of the intermaxillary gland, is supplied with scattered groups of mucous goblet cells.

Xenopus laevis

The intermaxillary gland of *Xenopus laevis* also belongs morphologically to Müller's specialized Type V but histologically and cytologically it is the least specialized of the three anural types examined. For example, considerable areas of the alveolar epithelium are ciliated, (Pl. 1, fig. 6) whereas in the frog and toad ciliated epithelium is confined to the terminal parts of the ducts. In the frog and toad the tubules are lined uniformly by glandular cells of the goblet type secreting PAS negative granules but in *Xenopus* there are two types of glandular cells. One, the more numerous, secretes PAS negative material in the form of fine granules and is probably comparable with the gland cells of the frog and toad, the other secretes much coarser granules of PAS negative material surrounded by a PAS positive reticulum. This second type of gland cell is found in small groups of some five or six cells here and there in the epithelium and is more reminiscent of the epithelium found in the intermaxillary glands of the caudate Amphibia (*vide infra*). With Azur A the fine granules stain blue and the coarse granules rather more mauve.

Sudan Black B produces a marked positive reaction with the material surrounding the large granules. The luminal content of the gland is a uniform colloid staining green with the ' PAS fast green ' technique.

Caudate Amphibia

The intermaxillary glands of the caudate Amphibia that have been examined have cells secreting PAS positive material distributed throughout the whole gland, even to the ends of the alveoli In the Salientia, on the other hand, PAS negative granules alone are secreted by the gland proper, and PAS positive material (mucin) is confined to the walls of the short ducts and the glandular orifices. The three species of urodeles examined show considerable variation in the histology of their intermaxillary glands but the actual secreting cells have very similar characteristics differing mainly in their disposition within the gland. They are all columnar cells and the secretory granules whether positive or negative to PAS are contained in large goblets. The nuclei of these cells in the salamander and warty newt are small, spherical or slightly oval and are basally placed in the cell. In the palmate newt these nuclei are also basal but are larger, and oval, with the long axis of the nucleus parallel with the cell axis.

Salamandra salamandra (Pl. 1, figs. 2 and 4)

The salamander shows the highest degree of specialization amongst the caudate species examined in that the tubular alveoli are lined by an epithelium the cells of which secrete either PAS negative or PAS positive substances. The ducts are distinguishable from the tubules by their lower epithelium. Ciliated epithelium appears not only in the terminal parts of the ducts, although it is here more profuse, but scattered ciliated cells may be found here and there throughout much of the gland. Such cells occur either singly or in small groups and are usually in close proximity to one or more mucous goblets (Pl. 1, fig. 4).

The PAS positive material is both mucin—the cells secreting it being typical unicellular mucous glands with a large goblet and a cone-shaped basal nucleus—and also PAS positive granules which appear pale mauve in the early stages of their formation, gradually taking on a deeper shade as they pass towards the free border of the cell before being ultimately discharged into the lumen of the gland. The cells producing PAS negative granules appear very similar to those just described except that their granules stain green instead of purple. Within the lumina of the tubes both green and purple staining secretions are detectable, although they may merge to give a greenish-violet colour. With Azur A the " green " granules as well as the luminal contents stain blue while the mucin goblets are either a faint pink or colourless. It is not possible to distinguish two kinds of granules in Azur preparations so one must assume either that the " purple " granules do not stain at all or else that they stain uniformly with the " green " granules.

With Sudan Black B also, granules and luminal contents are uniformly positive and mucin goblets negative.

Triturus cristatus

The warty newt differs from the salamander in that, while some cells appear identical with the mucous goblets of the salamander, the others are filled with granules of two kinds reacting positively and negatively respectively to PAS. From the condition of the warty newt alone it could be argued that the goblet cell full of mucoid secretion represents the final stage in the secretory life of the cell and that the other cells are merely juvenile stages. This view, however, is not supported by the condition found in the salamander (*vide supra*) or by the observation that the secretion within the lumina of the tubules may stain purple (PAS positive), green (PAS negative) or the greyish-violet produced by a mixture of both types. The glandular epithelium of the intermaxillary gland of the warty newt therefore shows a greater degree of paedomorphy than that of the salamander. With Azur A the granules stain intensely blue as also does the secretion in the lumina while Sudan Black B again gives a positive reaction with both as in *Salamandra*.

Triturus helveticus

The palmate newt possesses an intermaxillary gland which is different from that of either the salamander or warty newt. Thus there are no mucous goblet cells amongst the cells lining the crypts but PAS positive material is secreted by the gland. This material appears in the form of a reticulum surrounding the green (PAS negative) granules that otherwise fill the supra-nuclear region of the secretory cell. The luminal contents are granular or reticular, stain PAS positive, and do not appear to contain green colloidal material such as is found in the tubules previously described. When, however, the gland is stained with Azur A the granules within the cells stain intensely dark blue and the luminal contents also react positively but are slightly more mauve than the intra-cellular granules. The PAS positive material *within the cells* does not appear to stain with Azur A, so presumably the reticulum within the lumina of the tubules contains PAS negative granules the green colour of which is largely masked by a surface layer of PAS positive material when PAS is used but which is revealed with Azur A which does not stain the superficial material. Sudan Black B gives a weakly positive reaction.

Glands of the tongue

Rana temporaria

The original announcement by Lépine (1870) of the presence of amylase in the frog's mouth named the glands of the tongue as the source of this enzyme. Cytological examination of the frog's tongue shows gland cells of two kinds (Pl. 2, fig. 10). Those on the under side of the tongue and on the adjoining buccal membrane of the mouth floor are true mucous goblet cells embedded in a richly ciliated epithelium, while the upper (dorsal) surface of the adult tongue is much folded to form deep crypts which are lined by an epithelium secreting PAS positive granules in unicellular glands. The granules are of a kind apparently similar to the PAS positive granules secreted by the larval mucosa in its pre-metamorphic condition (*vide supra p.* 462). Although this

secretory product is referred to as " granules " for convenience, close examination shows that the " granules " arise with PAS positive surface layers surrounding a spherical core of PAS negative material.

The core in many cases appears to persist right up to the time of discharge, skin and core being discharged together but some granules appear to consist entirely of PAS positive material. Just how these should be interpreted is not clear—the core may never have been present or it may have been discharged prematurely. There is a distinct and constant shade difference between the colour of this PAS positive surface layer (bluish purple) and that of the true mucin secreted by the mucous goblet cells (reddish-purple) on the underside of the tongue and in the buccal mucosa. Although the physical character of the secretion of the glands on the upper surface of the tongue is clearly " mucoid " the secretion is not mucin in the strict sense or it would not show such a different reaction to the stain.

Tests, by the techniques used in the present study, show positive reactions for amylases but are negative for proteases and presumably the amylases are associated with the PAS negative core. The reaction of this core with toluidine blue (or Azur A) is slightly positive, or absent, in contrast to the positive orthochromatic reaction of the PAS negative granules in the intermaxillary gland. The appearance of the PAS positive material in the acini of the salivary glands of the male mouse is strictly comparable with the appearance just described for the frog's tongue except that in the mouse the PAS negative core is relatively larger and more persistent and accordingly the PAS positive reticulum never becomes completely " granular." In other words the " granules " appear to be duplex in character, although, as Baker (1957) rightly points out, such an appearance may in some cases be due to staining artifacts. It is hoped by histochemical studies to illuminate this point still further but in the meantime it must be noted that Azur A stains only the peripheral portion (purple) leaving the core unstained. This quite different stain reacting in a similar manner suggests that the granules are indeed duplex. In these respects the acini of the salivary gland of the mouse also resemble secretory cells found in the tongues of other Amphibia e.g. the toad and salamander (*vide infra*).

In the buccal mucosa of the pre-metamorphic tadpole of the frog numerous PAS positive granules may be seen which are strictly comparable with those of the glands of the adult tongue.

Bufo bufo

The toad's tongue, like that of the frog secretes mucin in typical goblets on its under surface while the upper surface is deeply folded with glandular crypts. The glandular cells lining these crypts show a PAS positive reticulum enclosing PAS negative granules staining greyish blue. These granules have only a very slight positive reaction (or none) with toluidine blue and a mauve colour with Azur A and thereby differ from the PAS negative granules of the intermaxillary gland (*vide supra*). The luminal contents of the crypts are mainly PAS positive but a few PAS negative particles may be seen here and there. The irregular shape and size of these however suggests that they may

originate from escaped lymphoctyes rather than from gland cells. Such lymphocytes can be seen here and there between the epidermal cells—presumably in transit. There are no PAS negative granules or any PAS negative colloid resembling the product of the intermaxillary glands or of the cells lining the crypts of the salamander's tongue (*vide infra*). The physical character of the tongue secretions of the toad is also very different from those of the frog and salamander, being at once less sticky and less copious. Under the 1/12 inch objective isolated cells, or small groups of cells, which contain green refractive granules, may be seen in the mucosa of the lower surface of the tongue and in the mucosa of the palate. These granules also react positively and orthochromatically with Azur A (MacNeal) and hence these granules could represent a source of proteases but no discharged granules, such as can be readily seen in the intermaxillary gland, can be detected.

Salamandra salamandra

The salamander's tongue (Pl. 1, fig. 7 and 8) presents a different picture. As mentioned above both green and purple granules may be demonstrated amongst its secretory products and its cytology is accordingly different. The glandular crypts are very deep and the glandular cells lining their walls do not present a uniform appearance. The cells deep in the bottom of the crypts (zone C, Pl. 1, fig. 7) produce green (PAS negative) granules, but very quickly as one passes up the sides of the crypts a purple (PAS positive) reticulum may be seen between the granules (zone B, Pl. 1, fig. 7), and the closer the surface of the tongue is approached the denser becomes this PAS positive reticulum and the fewer the PAS negative granules (zone A, Pl. 1, fig. 7). The reticulum is discharged into the lumen with the granules, the latter however are clearly visible under the 1/12 inch objective, both within the cells and amongst the luminal contents (Pl. 1, fig. 8). They merge with one another and produce green colloidal masses which, in turn, merge only very slowly with the PAS positive secretion and some may still be detected as distinct units amongst the secretion covering the surface of the tongue. The granules show a very similar reaction to those of the intermaxillary gland with Azur A. A noticable feature of the crypts of the salamander's tongue, particularly in their deeper portions, is the extensive vacuoles which separate the precipitated colloids from the free border of the cells (V. Pl. 1, fig. 8). There may well be fixation artefacts. They may appear elsewhere, e.g. in the intermaxillary glands of the frog (Pl. 1, fig. 3) but they are not invariably present in all the different glands investigated, so that the possibility that they may represent a soluble secretory product cannot be entirely excluded. As in the examples previously described the PAS negative material stains green and the PAS positive substance mauve. Mucous goblets occur on the lower side of the tongue and its adnexa, as well as here and there on the upper surface, but not down the sides of the crypts.

Triturus cristatus

The tongue of the warty newt resembles that of the salamander except that it is not so bulky, neither are the crypts so deep. Mucous goblet cells

occur quite plentifully on the upper surface of the tongue and also extend down the walls of the crypts and the reddish staining mucus is easily seen amongst the other luminal contents (purple and green). The PAS negative (green staining) material is relatively less plentiful than in *Salamandra ;* it shows similar reactions to Azur A. As noted above (p. 459) insufficient material was available to demonstrate conclusively by experimental means whether proteases are present or not.

Triturus helveticus

In the palmate newt the tongue is relatively much simpler than in the other two caudate types. The upper surface is folded but the folds are hardly deep enough to be called crypts and they, like the general surface of the tongue, contain mucous goblets as the sole type of gland cell. There is, however, a deep-seated (sub-hyoidean) tubular gland which discharges by means of ducts opening on to the upper surface of the tongue near its lateral margins. The cells of this gland secrete PAS positive granules staining bluish-mauve with PAS, and mauve with Azur A. They appear, in other words, to be comparable with the granules secreted by the cells lining the crypts of the frog's tongue. The luminal contents of this gland are colloidal and have similar staining reactions to the granules. There are no PAS negative granules within the cells and no green colloid within the lumen.

Buccal epithelium of Xenopus

Xenopus laevis is of course an aglossal toad. The mucosa lining the mouth is studded with single goblet cells secreting PAS positive material which stains more blue than true mucin and which also stains mauve with Azur A, and although the appearance of the secretion within the goblet is rather different from that in the lingual crypts of the types described above—for example there are no duplex granules—it seems probable that the secretion is more comparable with that produced in the tongue of other amphibia than with true mucin. Undoubted mucin producing cells are scarce in the anterior part of the mouth of *Xenopus*, taste buds, on the other hand are very numerous.

In summary, then, in addition to true mucin the oral glands of amphibia may produce either PAS negative granules only or with very little PAS positive reticulum (intermaxillary glands of frog, toad and *Xenopus*) or PAS negative granules surrounded by an extensive reticulum reacting positively with PAS (intermaxillary glands of urodeles and most tongues). The former type corresponds closely with the tubular portions and the latter type with the acinar portion of the submaxillary gland of the male mouse. Although so far no precise correlation can be made between the histological picture and the presence of either amylases and proteases it is nevertheless true that tissues secreting these enzymes in both mouse and amphibia possess cells that secrete PAS negative granules and it is hard to escape the inference that the granules may be associated with the enzymes either directly or as precursors. Whether a distinction may be drawn between such granules formed in a PAS positive reticulum and those which are not is less certain, but is must be noted that the granules of the intermaxillary glands (with or without a reticulum) react

strongly with Azur A and these glands secrete both proteases and amylases while most of the negative granules in the tongues (with a reticulum) react only weakly with Azur A, and in frog and toad at least, proteases are not associated with these. The general similarity between the overall picture in amphibia, both as regards cytology and the nature of the enzymes, and that presented by the submaxillary gland of the male mouse makes it clear that the significance of enzymes in the saliva must be sought well below the mammalian level in vertebrate evolution.

Ciliary tracts of the palate

Teleological explanations are out of fashion these days and it might be held pointless to look for any functional explanation of the relatively weak enzymes from the amphibian buccal glands reported here. Certainly their presence must be regarded as without significance so far as any direct effect on digestion is concerned. But, bearing in mind the elaborate and specialized nature of the glandular tissue associated with the presence of specific enzymes, it seems unreasonable to regard their presence as merely fortuitous and devoid of all significance. The ciliary tracts of the palate were therefore examined in order to discover whether they played any part in distributing these glandular secretions—particularly those of the intermaxillary gland—throughout the mouth. The secretion from the tongue, of course, bathes all the food more or less indiscriminately, but the openings of the intermaxillary glands, through which a secretion rich in proteases and amylases reaches the mouth, are always found just mesial to the internal nares and just anterior to the vomerine teeth. Any food brought into the mouth and lacerated or punctured by the vomerine teeth will immediately liberate its tissue fluids just where they will receive the maximum concentration of intermaxillary gland secretion. As first noted by Engelmann (1868) and later described in more detail by Merton (1923), the ciliary tracts on the palate of *Rana esculenta* are complex, both as regards length and rate of beat of cilia, but they are all related to the openings of the intermaxillary gland as a central point. The same is true for all amphibia examined in the present study, and although precise details vary considerably from one type to the next, it remains generally true that very strong ciliary currents radiate outwards in all directions from the openings of the intermaxillary glands and develop pathways bearing direct relation to the teeth (both palatal and marginal) and to the internal narial openings. These tracts, as determined by dropping colloidal graphite, or finely granulated charcoal, on the palate of a freshly pithed animal immersed in saline (0·75 per cent), are shown in Pl. 2, figs. 11 to 16. The relative strength of the ciliary currents is indicated approximately by the length of the arrow stems, the longer the stem the stronger the current. The openings of the glands in *Rana* can be seen clearly in Pl. 2, fig. 11 and it is quite obvious that all the really significant ciliary currents are related to them, the subsidiary tracts, like those crossing the floor of the orbits, being no more than cleansing currents. No ciliary tracts that actually lead into the internal nares have been observed although they pass right round the rims of these openings, but it is evident that such pressure changes as will occur during normal breathing movements in

the living animal must inevitably result in the flow of some materials carried by these ciliary tracts into and out from the nasal cavities. Further, the main concentration of taste buds on the palate of the frog occurs just along those ciliary tracts which are indicated as running around the inner margin of the upper jaw, and below the parasphenoid near the midline of the palate. As shown by McDonald *et al.* (1928) these cilia are under nervous control, being stimulated by the sympathetic and inhibited by the parasympathetic fibres.

In *Salamandra* (Pl. 2, fig. 12) there are definite ciliary tracts involving short but rapidly moving cilia leading *outwards* from the nasal sacs through the internal nares into the mouth. This outward current, however, involves only particles and fluid in close proximity to the mucosal surface, but it induces an inward compensatory current in the fluid occupying the more central regions of the narial openings and cavity. Granules of charcoal can be observed to enter the nose in this manner only to reappear later, moving slowly out from the nose in a strand of mucus closely applied to the mucosal surface. Thus there is in the salamander a definite interchange between nose and buccal cavity of fluid containing the secretion of the intermaxillary gland mixed with the general fluid contents of the mouth. The main ciliary tract leaving the nose carries the particles along the groove running from the outer margins of the internal nares towards the inner margins of the upper jaw. It will be noticed that strong ciliary tracts carry the secretion from the intermaxillary glands over the bases of the vomerine teeth and then posteriorly along the space between those two rows of teeth. Taste buds are plentiful along these tracts.

The warty newt (Pl. 2, fig. 15) shows a very similar state of affairs. It has similar compensation currents entering the nose and the main ciliary tracts are disposed in a manner very like those of the salamander.

The palmate newt (Pl. 2, fig. 16) differs in some respects from the salamander and warty newt. As in all Amphibia, strong ciliary currents radiate from the opening of the intermaxillary gland. Some pass to the margin of the upper jaw and proceed backwards to the pharynx just mesial to the maxillary teeth, others beat into the internal narial openings on their mesial aspect. Emerging from the nose laterally along the naso-buccal grooves, these ciliary currents ultimately join the maxillary tracts as shown in Pl. 2, fig. 16. There are short cilia between the palatine teeth but they produce only a very weak current which is not always easy to demonstrate. It is in no way comparable to the strong currents in this position in the other two urodeles examined. The current into the nose is a true ciliary current (not a compensation current as in *Salamandra*) and is much stronger in the palmate newt than in the salamander and warty newt and it would, therefore, appear that the nose, or more probably the vomero-nasal organ, is more important in this species, *vide infra*.

The permanently aquatic *Xenopus* (Pl. 2, fig. 13) shows an interesting modification which serves to emphasise the importance of the secretion from the intermaxillary glands. The openings of the glands are situated just antero-mesial to the nares, as in other amphibia, and the ciliary tracts diverge from them and carry the secretion around the narial openings and along the specially developed ciliated grooves which run from the outer extremities of

the nares, round the outher margins of the orbits to the throat. The ciliary current along these grooves is quite rapid and the grooves are richly supplied with taste buds. It is suggested that the significance of the grooves is in order that the glandular secretion from the intermaxillary glands, mixed with any material expressed from the food by the teeth in the front of the mouth, may reach the taste buds without being washed away, and without undue dilution. It will be seen that most of the ciliary tracts on the palate beat in such a direction as to ensure that any fluid in contact with them is ultimately carried into those naso-pharyngeal grooves and hence will mingle with the secretion from the intermaxillary glands already in the grooves.

The common toad, *Bufo bufo* (Pl. 2, fig. 14) on the other hand is edentulous on the upper jaw and here the ciliary tracts are well developed only in the immediate vicinity of the openings of the intermaxillary glands. The ciliary action is quite vigorous and would bring about a thorough mixing of secretion and buccal contents, but there are no well defined tracts leading from this area towards any sense organs. There is instead a general but relatively weak ciliary current moving backwards over the entire palatal surface together with a rather stronger one passing around the margins of the upper jaw. This latter current is really the continuation of the one passing from the gland openings around the internal nares. Bufo is the only amphibian studied in the present work that is without teeth on the palate to liberate tissue fluids from the food and it is the only example without well defined palatal ciliary currents to carry such fluids mingled with the secretion from the intermaxillary glands towards organs of olfaction or taste.

GENERAL RESUMÉ AND DISCUSSION

It has not been the purpose of the present work to attempt to elucidate the precise chemical nature of the several secretory products of the amphibian oral glands, but rather to establish the nature of the enzymes produced and to describe the tissues concerned with their production. It is hoped to pursue the histochemical line of enquiry and present the results in a later paper, but in the meantime this much may be said, the PAS positive reticulum found in the cells of the intermaxillary gland is also positive to Sudan Black B, and would therefore appear to be a lipid, and since the material was fixed in Bouin, probably a glycolipid or phospholipid (Pearse 1951), whereas that found in the cells of the lingual crypts does not stain with Sudan Black B although it appears quite similar when stained with PAS. It is fast to diastase and hyaluronidase and is therefore probably a muco-protein, the protein portion will react feebly with Azur A.

No satisfactory separation of the PAS positive granules secreted by the epithelial cells of the mucosa lining the mouth cavity of the frog's tadpole into two kinds, corresponding with the above distinction, has so far proved possible.

The pre-metamorphic epithelium of the tadpole thus becomes regionally differentiated at metamorphosis so that the several types of secretory products are found in different sites, in various specialized oral and lingual glands. Even in the case of mammals where both the histology and physiology of the various salivary glands have been extensively studied (cf. Babkin 1950)

no really satisfactory explanation seems forthcoming as to the true purposes served by salivary enzymes. Jenkins (1954) for instance says (p. 189) " Although salivary amylase is the only enzyme sufficiently active in the mouth to play any significant part in human digestion it is not the only enzyme present, as is often stated . . . Some of these enzymes may play an important part in processes which occur slowly and are highly localized (such as dental caries) but they may be ignored as far as the digestion and utilization of food is concerned."

The presence of buccal enzymes, secreted by specially elaborated glands, in all classes of terrestrial vertebrates from amphibia to mammals suggests that it may be worthwhile to think in terms of one common basic function which the salivary enzymes may serve, as distinct from various specialized purposes which may be served from time to time in particular cases. For example the venom of poisonous snakes is elaborated in specialized parotid glands, but this clearly is a secondary and not a primary purpose. Now truly aquatic vertebrates, such as fish (of various sorts) possess gustatory and olfactory receptors capable of detecting substances only in aqueous solution. Throughout the terrestial vertebrates this still applies to the gustatory sense while in animals such as amphibia and many reptiles, where Jacobson's organ retains its olfactory function (cf. Bellairs 1942) the olfactory sense may also be said to detect substances in solution.

This aspect of the question, so far as the human mouth is concerned is discussed by Jenkins (1954) on p. 213 as follows :— " The sensation of taste is produced only by substances in solution. Some foods such as fruits, contain such a high proportion of water that probably all the substances which have a taste are already in solution and their taste may be perceived as soon as they are released by mastication. Other foods, biscuits for example, contain relatively little water and before their taste becomes apparent saliva must dissolve out their flavoured constituents. By this means, saliva not only makes eating more pleasureable but may assist in the detection of unwholesome contaminants of food." Although these terms can hardly be applied to amphibia yet the buccal enzymes, by releasing flavoursome substances from the food may well serve to assist in food acceptance or rejection.

The hypothesis gains support when one considers the relation between the openings of the intermaxillary glands and other structures on the palate.

If the theory that the *primary* purpose of buccal enzymes is the liberation of substances capable of stimulating gustatory or olfactory sense receptors is substantiated then we may have at the same time a reasonable explanation for the apparently curious distribution of salivary amylase in mammals. Thus this enzyme is known to be absent or very weak in ungulate herbivors but present and strong in rodents. The juices of grasses and fresh vegetables are already rich in soluble sugars whereas the drier (grain) or woodier (stems and bark) food of rodents is not. Much further work, however, is required before any close correlation between diet and buccal enzymes could be made. It is true that amphibian buccal enzymes are relatively very weak but it must be remembered that some substances can be smelt (or tasted) in such extreme

dilution that in some cases at least the threshold of the sensory cell may well be a single molecule of the stimulating substance.

The suggestion of Hartridge (1945) may be of significance in this matter, namely that the lingual taste buds (of mammals) react alike to substances in the buccal fluids and in the blood and that imbalance between these two fluids in respect of sapid substances may produce an appetite or desire for them. Thus when the salt content of the blood is greatly decreased (as in Addison's disease) a craving for salt occurs together with an enhanced susceptibility of the taste buds for salt (cf. also Yensen, 1958 a & b). The " taste-bud average " so to speak can thus be maintained by high concentration in the mouth in spite of low concentration in the blood. In the same way higher than normal concentrations of such substances in the blood may make certain foods containing them nauseating. In this way Hartridge explains the instinctive appetites of wild animals which never seem to suffer from deficiency diseases. The suggestion made here is that buccal enzymes may make an important contribution to any such mechanism by releasing from the food a full range of soluble substances.

SUMMARY

The presence of an amylase in the saliva of the adult frog and toad is confirmed and is reported for the first time in the tadpole stages of the frog, from early internal gills onwards and also in the adult stages of *Xenopus laevis*, *Salamandra salamandra*, *Triturus helveticus* and *Triturus cristatus*.

Proteases are also reported for the first time in the buccal secretions of the frog and salamander.

Cells in the intermaxillary glands of the frog, toad, clawed toad, salamander and newt and in the tongues of some of these animals secrete granules that, by their staining reactions with PAS, Azur A (MacNeal) and Sudan Black B would appear to be of a similar nature to those secreted by the terminal portions of the intralobular ducts of the submaxillary gland of the male mouse. All these structures have been shown experimentally to secrete enzymes.

In the pre-metamorphic stages of the frog's tadpole granular secretions, both PAS positive and negative, are secreted by the general buccal mucosa, while the specialized glands characteristic of the adult—intermaxillary gland and the lingual crypts—are produced coincident with metamorphic changes.

Various histological differences are noted between the intermaxillary glands and tongues of the several species of Amphibia studied.

The relations between the openings of the intermaxillary glands and the ciliary tracts on the palate of the six species of Amphibia studied are described, and it is concluded that they serve to carry the enzyme-laden secretion of the intermaxillary glands mixed with food across the receptors of either the vomero-nasal organ and nose or the palatal taste buds or both.

The suggestion is made that the primary function of buccal enzymes is to liberate sapid and odoriferous substances from the food and thus form the basis of instinctive appetite as well as, perhaps, of acceptance and rejection of food substances.

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EXPLANATION OF PLATES

(All the figures 1–10 inclusive are untouched photomicrographs)

PLATE 1

Fig. 1—Transverse section of the intermaxillary gland of the frog, stained PAS, ×14. Note the absence of mucus or PAS positive secretion.

" 2—Transverse section of a portion of the intermaxillary gland of the salamander, stained PAS, ×44. Note the abundance of mucus.

" 3—Transverse section of a portion of one tubule from the intermaxillary gland of the frog, stained Azur A, ×790. Note the PAS negative granules in the cells and the colloidal secretion in the lumen, the two being separated by clear vacuoles.

" 4—Transverse section of a portion of one tubule from the intermaxillary gland of the salamander, stained PAS, ×820. Note mucus goblets, PAS negative granules, PAS positive granules and cilia.

" 5—A section through the submaxillary gland of the male mouse showing granules within the cells of a tubule (PAS negative) and an acinus (PAS negative core surrounded by a PAS positive reticulum). Stained Azur A, ×840.

" 6—Transverse section of a portion of one tubule from the intermaxillary gland of Xenopus, stained PAS, ×1000. Note the abundant cilia, the PAS negative granules and the small masses of PAS positive material at the luminal edge of some cells.

" 7—Transverse section of a portion of the tongue of the salamander stained PAS, ×96. Note the three secretory zones, (a) where the secretion is positive, (b) a mixed zone where both positive and negative secretions occur and (c) a basal zone where the secretion is predominently PAS negative.

" 8—The portion of figure 7 outlined by the white rectangle photographed at a higher magnification (×870), showing PAS negative granules and colloid and PAS positive colloid. Note also the vacuoles separating the colloids from the secreting cells.

PLATE 2

Fig. 9—A transverse section of the head of the frog's tadpole, hind-limb stage, 36 days after hatching to show the developing bud of the intermaxillary gland. Stained PAS, ×525.

" 10—A transverse section of the marginal portion of the frog's tongue. Stained PAS, ×280. Note the mucus goblets on the ventral surface and the cells secreting PAS positive granules on the dorsal aspect.

" 11—Photograph of the palate of the frog with the principal ciliary currents indicated by arrows, ×2·75. (In this and in subsequent figures the speed and strength of the ciliary currents are approximately proportional to the length of the arrow stems).

" 12—Photograph of the palate of the Salamander, ×5.

" 13—Photograph of the palate of the Xenopus , ×4.

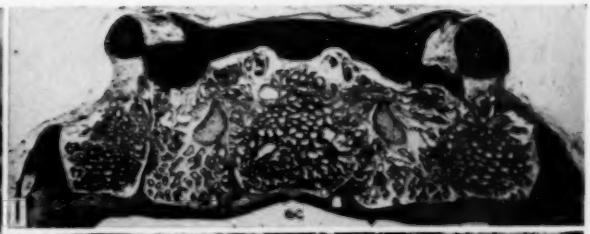
" 14—Photograph of the palate of the Toad, ×4.

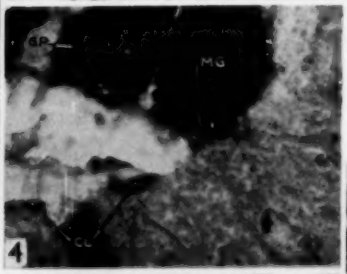
" 15—Photograph of the palate of the warty Newt, ×7.

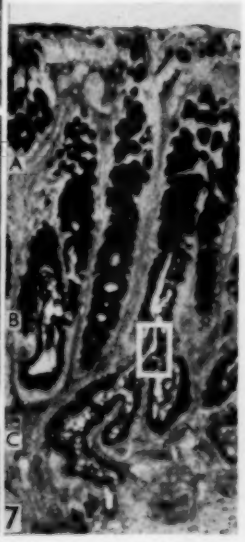
" 16—Photograph of the palate of the palmate Newt, ×9.

ABBREVIATIONS

A. Zone of salamander's tongue secreting PAS positive material.
AC. Acinus.
B. Zone of salamander's tongue secreting both PAS positive and PAS negative material.
BC. Buccal cavity.
C. Zone of salamander's tongue secreting predominently PAS negative granules.
CL. Cilia.
CN. Colloid secretion (PAS negative).
CP. Colloid secretion (PAS positive).
GN. PAS negative granules.
GP. PAS positive granules.
IMG. Anlage of intermaxillary gland.
MG. Mucus goblets.
T. Tubule.
V. Vacuole







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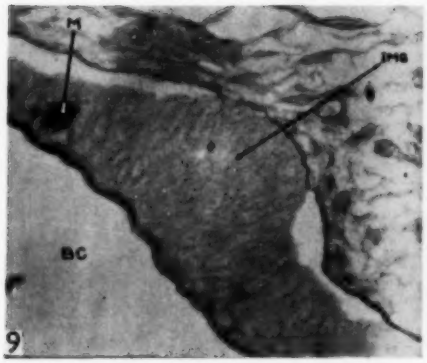
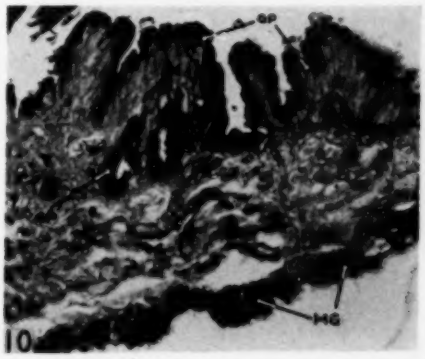
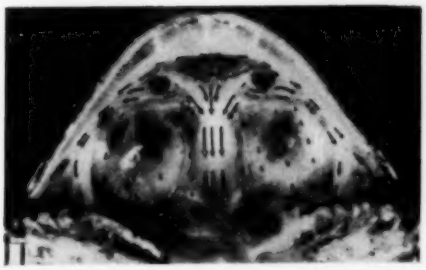


9



10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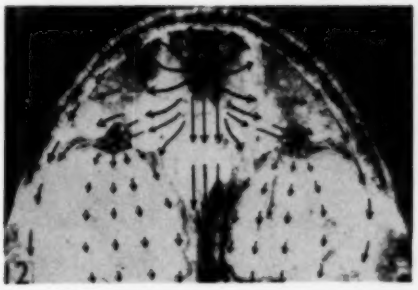
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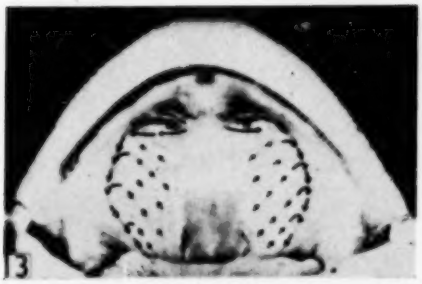
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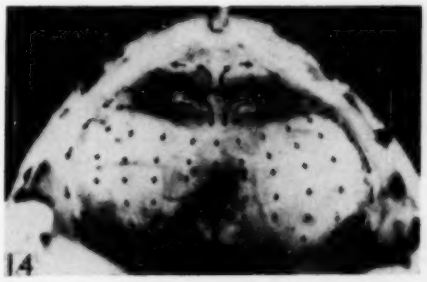
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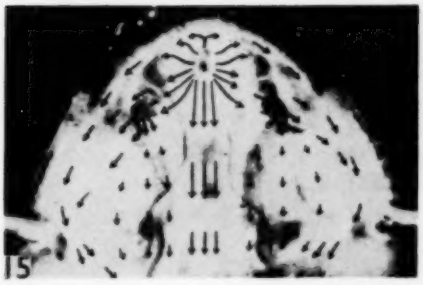
15

16

The sources and nature of salivary secretions in Amphibia.

AN OUTBREAK OF MALIGNANT CATARRH AMONG THE PÈRE DAVID DEER

BY

E. H. TONG, MARTIN SENIOR AND C. R. E. HALNAN

Whipsnade Park, Dunstable, Beds.

[Accepted 11th October 1960]

An outbreak of Malignant Catarrh was confirmed among a herd of Père David Deer (*Elaphurus davidianus*) at Whipsnade Park. This is of very great interest as although it is a common disease in Africa it is relatively unknown in Great Britain. This disease caused severe losses in the valuable herd at Whipsnade in 1959, but fortunately did not spread to the second herd of Père David nor to other ruminant species. Investigations did not reveal the presence of the disease among the gnu, which are commonly affected in Africa, nor any other species. The affected deer showed lesions of the alimentary tract, eyes and respiratory passages, together with a stiffness of the gait. Many secondary bacterial invaders were found at post mortem, but the extensive laboratory tests of the Ministry of Agriculture, Fisheries and Food Central Veterinary Laboratory at Weybridge showed by transmission experiments, that it had an incubation period of seventeen to twenty-three days and confirmed the disease to be Malignant Catarrh.

CONTENTS

	Page
The history of the outbreak	477
Preventative measures against spread of the disease	479
General herd treatment of the disease	479
Clinical and laboratory investigation	480
Pathological examination	480
Discussion	481
Acknowledgments	483

THE HISTORY OF THE OUTBREAK

In the early spring of 1959 an infectious disease, primarily affecting the mouth, appeared among the herd of Père David Deer (*Elaphurus davidianus*) kept in the Chartley Paddock at Whipsnade Park. The herd had been established in the same paddock for more than fourteen years, the original foundation herd being given by the late Duke of Bedford and hand reared at Whipsnade. During the succeeding years the herd had grown, partly by addition of more calves from Woburn, and latterly by the birth of calves to the hinds, until in 1957 it was decided to divide the herd into two so as to prevent over-stocking of the paddock. In addition to those required for the establishment of the herd a number of calves had been hand reared so that specimens could be sent overseas to other zoological gardens.

On the 14th April, 1959, a female Père David Deer was noticed standing away from the herd. This hind was about eight years old. She was not feeding, preferring to stand in the dew pond and although she dipped her head in water no apparent attempt was made to drink. When she moved her gait showed an apparent stiffening of hind quarters. It was impossible to catch the deer and death occurred early next morning.

A close watch was kept on the herd which grazed normally and appeared quite well. During this period four calves were born to the hinds. No sign of further illness occurred until the third week in May when another hind was reported ill. The clinical picture was very similar. She stood away from the herd and became distressed when attempts were made to catch her, respiration being markedly quickened. This animal also sought the pool and made chewing and licking motions with the mouth. The hind died forty-eight hours later on the 22nd May.

A review of the past history of the herd recalled a resemblance to the illness of a stag in the late spring of 1957 which lasted for a week. This animal showed some respiratory distress with a tympanic condition of the rumen and would seek water to stand in. It was impossible to attempt to catch the stag, but it recovered and returned to normal grazing after a week, remaining apparently quite well. A year later (1958), however, it showed similar symptoms and died—the post mortem showed broncho-pneumonia and septicaemia due to *Fusiformis necrophorus*. Shortly after, in the same year, a hind also died exhibiting similar symptoms and cause.

Returning to the outbreak of this year, nothing abnormal could be seen in the rest of the herd after 22nd May until the 16th June when a four-year-old female became ill and we were fortunate enough to catch her in the corner of the field and made a detailed clinical examination. Preliminary observation showed swelling of the lips, licking motions with tongue or sucking with corners of lips and blepharospasm of the eyelids. When made to move the hind quarters action was stilted. The mouth, anterior nares and eyes were closely inspected. Minute ulcers could be seen on the conjunctiva of the eyes and on the mucous membrane of the mouth. The tongue was somewhat furred. The lips were swollen and cracked at the commisures. The snout was hard and cracked, and there were cracks in the pedal epithelium of the web between the claws. The mucosa of the vulva was deeply injected. Temperature 101°. A tentative diagnosis that this was a mucosal disease was arrived at after examining the first live case. Treatment was undertaken by injecting 2 mega units of penicillin and 1 gm. achromycin intramuscularly. The prognosis was not promising as the hind weakened during the afternoon and it died the next morning.

The incidence of disease was apparently quickening as the fourth case was seen on Monday, 23rd June, and this female was successfully caught the same day, so again a detailed clinical examination was possible. The same clinical picture as in the previous case was obtained, but in this case the lips were considerably more swollen. Temperature 101°. In view of the bacterial findings in previous cases 100 c.c. of 33·1/3 per cent sulphamezathine solution was injected subcutaneously together with 1 gm. of achromycin intramuscularly. The prognosis was very guarded as this deer was very ill. A further gramme of achromycin was injected on Tuesday, death occurring late that night. Telephonic consultations were held with the Director of the Central Veterinary Laboratory and with the Department of Medicine of the Royal Veterinary College to obtain further assistance at the post mortem examination which took place on 25th June.

PREVENTATIVE MEASURES AGAINST SPREAD OF THE DISEASE

In view of the possibility of transmission of this disease to other ruminants in the Park precautions were taken early in the outbreak to protect the stock. No members of the staff were allowed into the Paddock or building, and a strict disinfection routine was carried out. Food was supplied to the deer through a hatch in the shelter in the paddock. Fortunately the Chartley Paddock is surrounded by road and paths so nearby deer and other ruminants could not come into direct contact. The observations kept on the herd indicated an infection which appeared to have an incubation period of approximately seven to ten days, for in the last two cases although they grazed and stayed with the herd a general dull appearance could be detected prior to the onset of actual illness.

GENERAL HERD TREATMENT OF THE DISEASE

To combat the bacterial infections found at post mortems an attempt was made to treat the animals in the paddock. Sulphamezathine was chosen as many of the organisms found respond to treatment with this drug. One pound of powder was mixed with fifteen pounds of salt and placed in small heaps under trees. In addition six litres of the 33·1/3 per cent solution was mixed in the drinking trough which held about sixty gallons of water. This was, however, so unpalatable that the animals refused to drink and clean water had to be supplied. The dew pond in the meantime had dried out and only had a little mud in the centre. In view of Case 2 and of the possibility of a fly borne infection this was fenced off and sprayed with gamma benzene hexachloride (Lorexane).

The Paddock was also divided into two parts by a five foot chain link fence from east to west and the deer confined to the north side.

On Wednesday, 1st July, the fifth case, another female, was seen and she was caught the same day and put in the stable. Accompanied by the Society's Pathologist and Mr D. Johnston, Divisional Veterinary Officer, of Bedford, we were able to make a detailed examination, the clinical symptoms being identical with the previous cases. It was generally agreed that the nearest approach appeared to be a type of mucosal disease. It should be mentioned that the possibility of foot and mouth disease had been considered at the beginning, but was ruled out as the lesions were definitely not in any way typical of this disease.

It was decided to use chloramphenicol (chloromycetin) for this case and 2 gm. in 5 ml. were injected intramuscularly daily. The deer improved and began to drink and ate grass provided. By Saturday the mouth appeared almost normal. Five doses of chloramphenicol were given, but on the occasion of the last dose (Sunday) the deer appeared a little duller although she was eating and drinking. On Sunday afternoon the keeper observed she was standing in the drinking receptacle and she died suddenly in the night.

About this time the herd stag seemed to be a little off-colour for one day but nothing developed and he returned to normal grazing and activity.

CLINICAL AND LABORATORY INVESTIGATIONS

The help of the Central Veterinary Laboratory was again sought and they asked for a live case to be sent to them so that they could make a complete clinical and post mortem examination. On the 8th July a suspected case was caught up, a four-year-old female, and a few tiny ulcers were noted on gums and snout. This hind was sent to Weybridge and proved to be the earliest case we succeeded in catching. It is of great interest that all the successful transmissions that the Central Laboratory have made were from this animal.

On the 9th July two more cases, a hind and a stag, were caught up, and on Friday, 10th July, Mr H. I. Field, M.Sc., M.R.C.V.S., of the Central Veterinary Laboratory visited Whipsnade Park and accompanied by Senior made a detailed survey. He was able to closely observe the two cases and was given a detailed clinical outline illustrated by colour slides of the lesions both in the living animal and at post mortem.

The stag broke out of the yard after a few days and rejoined the herd; the female died on the 17th and was sent to Weybridge. The stag stayed with the herd, but was found down in the yard on Sunday, 25th July, and died. This was also sent to Weybridge.

After the preliminary report from the Central Laboratory, in which contagious malignant catarrh was considered as a possible alternative to mucosal disease, several Red Deer were sent to Weybridge for transmission experiments, and successful passages both in these deer, giving typical symptoms in seventeen to twenty-three days, and in calves at thirty-five days proved that the disease in the Père David Deer was the viral infection known as malignant catarrh.

Shortly after sending the third specimen to Weybridge the remaining stags and the four calves were shot and the carcases burned. These deer had shown no evidence of illness throughout the duration of the epidemic, and no other ruminants in the Park have shown evidence of the disease.

The Paddock was left empty for three months and all buildings, drinking receptacles, etc., thoroughly cleaned and disinfected. Subsequently Przewalski horses and Shetland ponies were kept in the two separate portions, and later the Chartley cattle replaced the Shetlands. Twelve months have passed since the onset of the disease but no evidence of the infection has reappeared.

PATHOLOGICAL EXAMINATIONS

Case 1. 10th April, 1959. An eight years old female. The snout was dry and hard and the lips thickened. The buccal cavity showed a dirty white necrotic material coating the inner aspect of the lips, gums, tongue and hard palate, extending backward to the pharynx. The underlying tissues were deeply inflamed and ulcerated. The lungs showed pneumonic congestion. The contents of the rumen and reticulum were exceedingly dehydrated and formed into spherical masses of varying sizes. The intestinal tract showed little apparent change. On bacteriological examination *Salmonella tyrphimurium* was isolated.

Case 2. 23rd May, 1959. A seven years old female. The mouth lesions were very similar to Case 1, but also numerous circumscribed raised plaques of a reddish purple colour, many denuded of hair, were found on neck and anterior shoulder region. Two similar lesions found near tail. They resembled lesions which could have been caused by biting flies, etc. The epithelium of the web between the cleys showed vertical cracking with some desquamation—there were no lesions on the coronets of the feet. The rumen and reticulum again showed the marked dehydration with formation of sperical masses. The bacteriological examination showed the presence of a non-haemolytic streptococcus.

Case 3. 17th June, 1959. A four years old female. The lesions of the head were more severe and the whole of the buccal cavity was deeply inflamed and coated by the thick necrotic exudate. Examination of the eyelids showed early ulceration of the conjunctiva. The respiratory and alimentary systems exhibited the same lesions as the first two cases, together with some early inflammatory congestion of parts of the intestinal tract. From the eye of this hind *Stapholococcus pyogenes aureus* was isolated.

Case 4. 26th June, 1959. A three years old female. The mouth lesions were very severe and extended into pharynx. Ulceration of the conjunctiva was again observed. A very marked pneumonic congestion. The contents of the rumen and reticulum showed the usual dehydrated condition. The mucus surface of the abomasum was slightly ulcerated and the small intestine showed a degree of patchy inflammation with some evidence of early ulceration. Bacteriological examination showed the presence of *Pasturella multocida* and Friedlander's *Pneumococcus* (*Klebsiella*).

Case 5. 5th July, 1959. A five years old female. The same severe lesions of the buccal cavity as in Case 4 were found, together with severe necrotic tracheitis and bronchitis. The alimentary tract lesions were more marked especially in the abomasum.

DISCUSSION

In all cases the most constant lesion was that of the buccal cavity, the dirty white necrotic exudate being formed by the sloughing of the mucous membrane due to the inflammation and ulceration of the parts of the mouth. The cracking of the horny epithelium of the snout and in the web of the feet was also a constant lesion.

It is of note that the lesions of the mouth became progressively more severe as the disease progressed and that the lesions progressively extended along the alimentary tract resulting in inflammation and ulceration of the stomach and intestine of later cases. The curious condition of the contents of the first and second stomachs was undoubtedly produced by severe dehydration of the body due to the inability of the deer to drink owing to the intense soreness of the mouth.

The diagnosis of the disease in the field is based upon the clinical picture, and course of the disease, together with the pathological lesions and epidemiology. Considering the most striking points of these we have :—

(*a*) *Clinical picture.*—Dullness, outstretched neck and lowered head, licking of lips, blepherospasm, anorexia, stiffened gait, desire to immerse muzzle and feet in water.

(*b*) *Course.*—Increasing depression with separation from the remainder of the herd leading to prostration and death in two to seven days.

(*c*) *Pathology.*—There may have been an early pyrexia, but during the course of the disease temperatures of 101° were recorded. The extensive lesions of the mouth with the sloughing of the mucous membrane from the inflamed tissues. The extension of lesions on to the muzzle and external nares. Small punctate ulcers on the eyelids extending on to the conjunctiva with a small amount of purulent discharge at the median canthus. In later cases the mouth lesions spread to the pharynx and respiratory system. The condition of the contents of the stomachs together with the inflammation and ulceration of the alimentary tract. The feet exhibiting cracking of the epithelium of the web.

(*d*) *The epidemiology.*—The incubation period was difficult to define as in the early stages periods of one month were observed between cases, but they occurred more frequently in later stages. If we consider the case of the stag found ill on the 9th July sixteen days elapsed before it died on the 25th July: This would give an approximate period of eighteen to twenty-one days as a possible course of the disease. The spread of the disease through the herd was not rapid by standards of virus disease but the mortality was 100 per cent.

(*e*) The bacteriological findings in these five cases were undoubtedly secondary or even post mortem, invaders, for in no case was a common bacterium found in any subject.

Reviewing the broad characteristics of the disease led to the consideration of known virus infections of bovines in this country.

Foot and Mouth Disease did not fit either the clinical symptoms or post mortem lesions. There were no vesicles in the mouth, the feet did not exhibit the characteristic lesions and the rate of spread in the herd was not typical.

Vesicular Stomatitis has a very short incubation period and is normally confined to the mouth, and again did not correspond with the lesions found.

Rinderpest and Blue Tongue were also possibilities but there were no importations that could bring these diseases and the lesions were not conclusive to either of these diseases.

Malignant Catarrh was a possibility but as it is so rare in England the *Mucosal Disease complex* seemed to be the most likely of the group to fit the overall picture and outbreaks of this disease have been reported from various parts of the country. The lesions of mucosal disease affect the mouth and also the intestinal tract with an incubation period of approximately twelve days, and these considerations led in the early stages of the outbreak to a tentative diagnosis of a mucosal disease affecting the Père David Deer.

The investigation by the Central Veterinary Laboratory eventually determined that the disease was malignant catarrh but the source of the infection has been impossible to trace.

ACKNOWLEDGMENTS

To Mr R. N. T-W-Fiennes, for post mortem and bacteriological help; Professor H. Burrows, of the Department of Medicine, Royal Veterinary College, for the assistance provided by Messrs Price, Dunlop and Lewis, at post mortems of Cases 4 and 5; Mr D. Johnston, Divisional Veterinary Officer, Animal Health Division, Ministry of Agriculture, Fisheries & Food, Bedford, for help in clinical and post mortem examinations; Mr J. E. N. Sloan, for parasitological investigation; Dr A. O. Betts of the Cambridge School of Veterinary Medicine for virology examinations; Mr J. N. Ritchie, Chief Veterinary Officer, Ministry of Agriculture, Fisheries and Food and Messrs P. J. Allsop, R. H. Ewart, H. I. Field, R. A. Huck, A. B. Patterson, and A. Shand, Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, Weybridge, for laboratory investigation.

NOTES AND ABSTRACTS

MEETINGS OF THE SOCIETY FOR SCIENTIFIC BUSINESS

8th November 1960

PROFESSOR A. J. E. CAVE, M.D., D.Sc., F.R.C.S.,

in the Chair

REPORT ON THE ADDITIONS TO THE SOCIETY'S MENAGERIE: OCTOBER 1960

By L. HARRISON MATTHEWS, M.A., Sc.D., F.R.S.

The registered additions to The Zoological Society's Menagerie in London during the month of October were 97 in number. The following are of special interest :—A jungle cat *Felis chaus* presented by Mr J. A. Looker ; a grey monitor *Varanus griseus* presented by Major J. H. Muscat ; a Blanding's snake *Boiga blandingi* and two red-and-black skinks *Riopa fernandi* presented by Dr G. T. Dunger. A male jaguar *Panthera onca* and a male coyote *Canis latrans* were purchased to make up suitable breeding pairs of these species and other purchases included three Humboldt's penguins *Spheniscus humboldti*, two thick-billed green pigeons *Treron curvirostra nipalensis*, a chestnut-breasted finch *Munia castaneothorax*, a speckled-fronted finch *Sporopipes frontalis*, two grey-headed lovebirds *Agapornis cana*, two red-faced lovebirds *Agapornis pullaris* and a collection of chameleons including fourteen *Chamaeleo bitaeniatus ellioti* and nine *Chamaeleo bitaeniatus hoehnelii*, new to the collection. Two Barrow's golden-eyes *Bucephala islandica*, two bob-white quails *Colinus virginianus*, two partridges *Perdix perdix perdix*, a black-headed oriole *Oriolus xanthornus xanthornus* and two tambourine doves *Tympanistria tympanistria fraseri* were received in exchange. Two pumas *Felis concolor* and a Timor deer *Cervus timorensis* were born in the Menagerie ; the pumas, born in the North Mammal House, were sired by the same male as the pair born in the Lion House earlier in the year but have a different mother. The male has now been returned to the Lion House where it is hoped he will mate again with the female there. A great grey tinamou *Tinamus tao* was hatched. During the same period there were 16 additions to the Menagerie at Whipsnade Park, including some domestic animals and excluding transfers from London. The following is of special interest :—a bison *Bison bison* born in the Park.

The following communications were read and discussed :—

Mr R. N. T-W-FIENNES. Comparative anatomy of the posterior vena cava with reference to the mechanisms of shock.

Dr THELMA ROWELL. On the retrieving of young and other behaviour in lactating golden hamsters. [To be published in the " Proceedings "].

Dr PHILIP S. CORBET. The feeding habits of non-cichlid fishes in the Lake Victoria basin, with remarks on their adaptation to lacustrine conditions. [To be published in the " Proceedings "].

13th December 1960

PROFESSOR J. E. SMITH, M.A., SC.D., F.R.S.,
Vice-President, in the Chair

REPORT ON THE ADDITIONS TO THE SOCIETY'S MENAGERIE : NOVEMBER 1960

By L. HARRISON MATTHEWS, M.A., SC.D., F.R.S.

The registered additions to The Zoological Society's Menagerie in London during the month of November were 105 in number, including some domestic animals. The following are of special interest :—An ornate spider monkey *Ateles geoffroyi ornatus* Gray was presented by Mr I. W. Sport; three forest wagtails *Dendronanthus indicus* Gmelin and two sooty flycatchers *Muscicapa sibirica* Gmelin were presented by Messrs G. H. and J. R. Newmark. All these specimens are new to the collection. A female silver-grey phalanger *Trichosurus vulpecula* was presented by Mr Michael J. Tyler (who presented a male earlier this year) ; a male cotton-headed tamarin *Oedipomidas oedipus* was purchased to mate with the female presented last year and other purchases included a pair of red-mantled tamarins *Leontocebus illigeri* with their baby ; three Humboldt's penguins *Spheniscus humboldti* and six banded basilisks *Basiliscus vittatus* ; exchanges included an olivaceous woolly monkey *Lagothrix cana olivacea* (Spix) and a Derbian opossum *Caluromys derbianus* (Waterhouse) both new to the collection, a pair of white-headed sakis *Pithecia pithecia*, a pair of bobcats *Felis rufa* (last exhibited in 1938) and a male white-headed sloth *Choloepus hoffmanni capitalis* to mate with the female purchased earlier this year. Five boa constrictors *Constrictor constrictor* and a de Brazza's monkey *Cercopithecus neglectus* were born in the Menagerie ; this is the first time the birth of a de Brazza's monkey has been recorded here and there was considerable disappointment when the mother deserted her baby, but a member of the Monkey House staff volunteered to hand rear it and so far all is going well. During the same period there were 29 additions to the collection at Whipsnade Park, including some domestic animals and excluding transfers from London. The following are of special interest :—twenty white storks *Ciconia ciconia* and three black storks *Ciconia nigra*, received in exchange and two collared peccaries *Tayassu tajacu* and two nilgai *Boselaphus tragocamelus*, born in the menagerie.

The following communications were read and discussed :—

Dr D. B. CARLISLE. Locomotory powers of adult ascidians. [To be published in the " Proceedings "].

Dr H. J. DE S. DISNEY, Dr B. LOFTS and Professor A. J. MARSHALL. Photostimulation in the red-billed dioch. [To be published in the " Proceedings "].

Dr G. FRYER. New light on problems of speciation in African lakes. [To be published in the " Proceedings "].

NOTICE

[" Proceedings ", Vol. 136, Part 2, was published on 10th March, 1961.]

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No. 5 VERTEBRATE LOCOMOTION. Proceedings of the Symposium held on 9th November, 1960.

No. 6 ASPECTS OF DISEASE TRANSMISSION BY TICKS. Proceedings of the Symposium held on 8th March, 1961.

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LIST OF CONTENTS

Vol. 136

Part 3 (pp. 301–487)

Page

Bowden, Ruth E. M. and Scheuer, J. L.
Comparative studies of the nerve supply of the larynx in eutherian mammals. (4 plates) 325

Burdon-Jones, C. See De Silva, P. H. D. H.

Cave, A. J. E.
The frontal sinus of the gorilla. (1 plate and 6 figures) 359

Davies, R. G.
The postembryonic development of the female reproductive system in *Limothrips cerealium* Haliday (Thysanoptera : Thripidae). (14 figures) 411

Davis, P. E. See Delany, M. J.

De Silva, P. H. D. H. and Burdon-Jones, C.
A new genus and species of leech parasitic on the fish *Cottus bubalis*. (1 plate and 5 figures) 343

De Silva, P. H. D. H. and Kabata, Z.
A new genus and species of leech parasitic on *Drepanopsetta platessoides* (Malm), the long rough dab. (3 figures) 331

Delany, M. J. and Davis, P. E.
Observations on the ecology and life history of the Fair Isle field-mouse *Apodemus sylvaticus fridariensis* (Kinnear). (5 figures) 439

Ewer, R. F.
The anatomy of the anomodont *Daptocephalus leoniceps* (Owen). (5 plates and 10 figures) 375

Fox, H.
Quantitative analysis of normal growth of the pronephric system in larval Amphibia. (1 plate and 10 figures) 301

Francis, E. T. B.
The sources and nature of salivary secretions in Amphibia. (2 plates and 1 figure) 453

Goodbody, Ivan.
Continuous breeding in three species of tropical ascidian. (6 figures) 403

Halnan, C. R. E. See Tono, E. H.

Hayman, R. W.
The Red goral of the North-east Frontier Region. (1 plate) 317

Kabata, Z. See De Silva, P. H. D. H.

Scheuer, J. L. See Bowden, Ruth E. M.

Senior, Martin. See Tono, E. H.

Silva, P. H. D. H. De. See De Silva, P. H. D. H.

Tono, E. H., Senior, Martin and Halnan, C. R. E.
An outbreak of malignant catarrh among the Père David deer 477

Notes and Abstracts
Report on the additions to the Society's Menagerie October, 1960 by L. Harrison Matthews, p. 485 ; Report on the additions to the Society's Menagerie November, 1960 by L. Harrison Matthews, p. 486.

NOTICE

"Proceedings" Vol. 136 Part 2 was published on 10th March, 1961.